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ABSTRACT

This book represents the proceedings of the Conference on Advancing Research on Developmental Plasticity: Integrating Behavioral Science and the Neuroscience of Mental Health. The conference featured scientific presentations from many leading scientists in behavioral sciences, neuroscience and psychiatry, as well as a poster session for newer investigators and roundtable seminars for more in-depth discussion. The conference focused on three primary areas of development: stress and early development, cognition, and social behavior. The primary goal of the conference was to bring together scientists from neuroscience, the behavioral sciences, and psychiatry in order to begin identifying substantive topics that may benefit from more integrated cross-disciplinary research on developmental plasticity. The proceedings emphasize the importance of developmental plasticity in brain and behavior to mental health. Identifying the mechanisms and the timing by which these mechanisms operate could produce a greater understanding of the multiple and interconnected levels of functioning that lead to adjustment, as well as a much better understanding of when and how functioning becomes impaired. By examining the natural developmental timing of sensitive periods for plasticity, scientists will have firmer data to inform decisions about when to implement prevention or intervention. Contains 19 presentations, 6 roundtable seminars, and 37 poster presentations. (Author/GCP)

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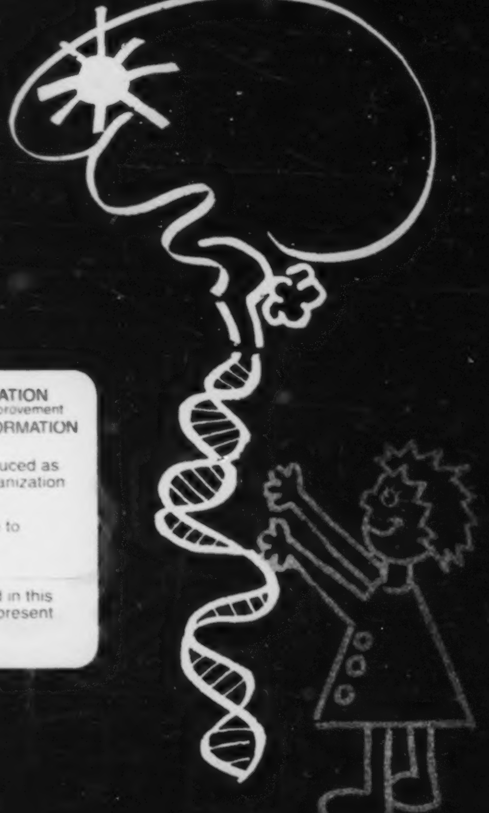
Advancing Research on Developmental Plasticity

Integrating the Behavioral Science and Neuroscience of Mental Health



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Advancing Research on Developmental Plasticity

Integrating the Behavioral Science and Neuroscience of Mental Health

Editors:

Della M. Hann
Lynne C. Huffman
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Preface

The National Institute of Mental Health (NIMH) is responsible for supporting research that aims to improve diagnosis and treatment of mental illness. Mental illnesses are diverse and involve many different behaviors. Similarly, the brain processes underlying these behaviors are themselves complex and in many cases poorly understood. One approach to advancing research on these themes is to promote integration of investigators from diverse perspectives to determine where there are common principals and concepts.

Beginning in 1995, a steering committee composed of NIMH staff and eight outside experts began discussions on the topic of developmental plasticity as a central theme for integrated discussions. Developmental plasticity was a particularly attractive theme because it is a challenge to the nature-nurture conceptions of development. In the past, it was assumed that environmental events determined only the psychological components of development (e.g., memory, habits, attitudes), while brain development progressed by means of fixed biological processes. The steering committee recognized that recent advances in developmental plasticity demonstrate the mutual influences of biology and environment in shaping the development of brain and behavior. To explore this topic more fully, the Conference on Advancing Research on Developmental Plasticity: Integrating the Behavioral Science and the Neuroscience of Mental Health, was held on May 12-15, 1996, in Chantilly, Virginia. This book represents the proceedings of the conference.

The Conference featured scientific presentations from many leading scientists in the behavioral sciences, neuroscience, and psychiatry, as well as a poster session for newer investigators and roundtable seminars for more in-depth discussion. Three broad substantive areas were explored: Stress and Early Development, Cognition, and Social Behavior. Each area was explored from the perspectives of the different represented disciplines, which at a minimum involved molecular and cellular neuroscience, behavioral neuroscience, behavioral science, and psychiatry. This volume of proceedings demonstrates the breadth of scientific perspectives represented at the conference and serves as a summary of the scholarly integration presented by each speaker.

A primary goal of the Conference was to assess the degree of scientific enthusiasm and readiness for pursuing cross-disciplinary programs of research on topics relevant to developmental plasticity. To a great extent, the Conference succeeded as an educational forum. For example, it emphasized the growing recognition in the scientific community that in order to answer many of the challenging questions in mental health and disease, it will be important to build bridges between the behavioral sciences, neuroscience, and psychiatry. It also highlighted how building bridges between these scientific domains can be quite challenging. Each area of science—be it neuroscience, behavioral science, or psychiatry—has a rich tradition and distinctive ways of conducting science, similar to a culture of science. In building bridges that span these diverse cultures, we need to find ways of fostering an atmosphere of openness that allows each culture to retain its unique richness while accepting, and hopefully, learning from others. Common interests will become the focal points in developing research agendas that help build the bridges and capitalize on the strengths and diversity of approaches from each scientific perspective to help answer some of our most challenging questions in mental health.

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National Institute of Mental Health
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Advancing Research on Developmental Plasticity: Integrating the Behavioral Science and Neuroscience of Mental Health

Chantilly, Virginia
May 12–15, 1996

Sunday, May 12	**WELCOME AND INTRODUCTIONS**
	Stephen H. Koslow (Director, Division of Neuroscience and Behavioral Science, NIMH)
	KEYNOTE ADDRESS
	David Kupfer (University of Pittsburgh Medical School) Developmental Plasticity: Is It the "Plastics" of the 90s?
Monday, May 13	**SYMPOSIUM I: STRESS AND EARLY DEVELOPMENT**
8:00 - 12:30 p.m.	*Chair*: Lynne Huffman (Division of Neuroscience and Behavioral Science, NIMH)
8:00 - 8:40	Martha Constantine-Paton (Yale University) Activity Dependent Synaptogenesis
8:50 - 9:30	Bruce McEwen (Rockefeller University) Hormones as Regulators of Brain Development
10:00 - 10:40	Eugene Emory (Emory University) Behavioral Development in Perinatal Life: Basic Principles
10:50 - 11:30	Dante Cicchetti (University of Rochester) Early Experience, Emotion, and Brain: Illustrations From the Developmental Psychopathology of Child Maltreatment
11:40 - 12:10	*Discussant*: Martha McClintock (University of Chicago) Concepts and Metaphors for a Multidisciplinary Approach to Developmental Plasticity
1:30 - 1:45	Perspectives From the NIH Office of Behavioral and Social Sciences Research

Monday, May 13	**SYMPOSIUM II: COGNITION**
1:30 - 6:00 p.m.	*Chair:* Douglas Meinecke (Division of Neuroscience and Behavioral Science, NIMH)
1:45 - 2:25	Pasko Rakic (Yale University) Cellular and Molecular Mechanisms of Cortical Development: Relevance to Congenital Cortical Malformations
2:35 - 3:15	Albert Galaburda (Harvard University) Propagation of Developmental Errors Accounts for Perceptual and Cognitive Deficits in Learning Disability
3:45 - 4:25	Elizabeth Bates (University of California, San Diego) Neural Plasticity in Humans: Evidence From Children With Early Focal Brain Injury
4:35 - 5:15	Allan Reiss (Kennedy Krieger Institute) Behavioral Neurogenetics Research: A Method for Analyzing Linkages Among Gene, Brain, and Behavior
5:25 - 5:55	*Discussant*: William Greenough (University of Illinois) Synapse Stabilization and Fragile X Protein Synthesis in the Rodent Brain
7:30 - 9:00 p.m.	**Poster Session: New Investigators**
Tuesday, May 14	**SYMPOSIUM III: SOCIAL BEHAVIOR**
8:00 - 12:30 p.m.	*Chair*: Israel Lederhendler (Division of Neuroscience and Behavioral Science, NIMH)
8:00 - 8:40	Joseph Coyle (Harvard University) Toxic Environments, Neuronal Plasticity, and Delinquency
8:50 - 9:30	Myron Hofer (Columbia University) Shaping Forces Within the Early Parent-Infant Relationship
10:00 - 10:40	Robert Emde (University of Colorado Health Sciences Center) Early Emotional Development: Integrative Perspectives From Longitudinal Study
10:50 - 11:30	James Leckman (Yale University) Maladies of Love—An Evolutionary Perspective on Some Forms of Obsessive-Compulsive Disorder

11:40 - 12:10 *Discussant:* Robert Cairns (University of North Carolina, Chapel Hill)
Developmental Plasticity and Continuity in Social Interactions: Attachment and Aggression

1:30 - 3:30 **ROUNDTABLE SEMINARS**

Stress and Early Development

Chair: Eugene Emory (Emory University)
Biobehavioral Profiles Related to Stress Reactivity and Ethnic Variation

Discussants: Dante Cicchetti, Lynne Huffman, Martha McClintock, Bruce McEwen, Martha Constantine-Paton, and Steven Zalcman

Cognition

Chair: Fernando Nottebohm (Rockefeller University)
Neuronal Replacement and Segmented Memories

Discussants: Elizabeth Bates, Albert Galaburda, Dennis Glanzman, William Greenough, Douglas Meinecke, and Allan Reiss

Social Behavior

Chair: Mary Rothbart (University of Oregon)
Temperament and Plasticity in Childhood

Discussants: Robert Cairns, Joseph Coyle, Robert Emde, Myron Hofer, James Leckman, Israel Lederhendler, and Mary Ellen Oliveri

7:30 - 9:30 p.m. **PANEL DISCUSSION: Roundtable Highlights and Recommendations**

Chair: Della Hann (Division of Neuroscience and Behavioral Science, NIMH)

7:30 - 8:00 Eugene Emory (Emory University)

8:00 - 8:30 Fernando Nottebohm (Rockefeller University)

8:30 - 9:00 Mary Rothbart (University of Oregon)

9:00 - 9:30 Group Discussion

Wednesday, May 13	**MASTER LECTURES: DEVELOPMENTAL PLASTICITY—INTEGRATION OF MOLECULAR, ORGANISMIC, AND SOCIAL PROCESSES**
8:00 - 12:30 p.m.	*Chair*: Della Hann (Division of Neuroscience and Behavioral Science, NIMH)
8:00 - 8:40	Robert Hinde (Cambridge University) Integrating Across Levels of Complexity
8:50 - 9:30	Helen Neville (University of Oregon) Variability in the Effects of Experience on the Development of Cerebral Specializations: Insights From the Study of Deaf Individuals
10:00 - 10:40	Felton Earls (Harvard University) Recovery From Profound Early Social Deprivation
10:50 - 11:30	Patricia Goldman-Rakic (Yale University) The Plasticity-Specificity Conundrum
11:40 - 12:30	**CONCLUDING REMARKS** Steven Hyman (Director, NIMH)

Developmental Plasticity Conference: Introduction

Della M. Hann

National Institute of Mental Health

Recent advances in our understanding of neural and behavioral development are dramatically changing the way we conceptualize and study brain development and psychopathology. Mechanisms of plasticity increasingly are recognized as being integral in the elaboration of brain structure and function during development. The ramifications of plasticity are profound: brain development that was once thought to be deterministic is now viewed as malleable and at times dependent on stimulation available in the environment. As such, research on developmental plasticity provides an unprecedented opportunity for investigators in neuroscience, the behavioral sciences, and psychiatry to address topics of similar interest and focus on the mechanisms that regulate the plasticity of typical and atypical brain and behavioral development.

Recognizing this opportunity, the National Institute of Mental Health (NIMH) Division of Neuroscience and Behavioral Science, the National Institutes of Health Office of Behavioral and Social Sciences Research, and the Developmental Plasticity Steering Committee developed and convened the NIMH conference on "Advancing Research on Developmental Plasticity: Integrating the Behavioral Science and the Neuroscience of Mental Health," May 12-15, 1996, in Chantilly, VA. A primary objective of the conference was to bring together scientists from neuroscience, the behavioral sciences, and psychiatry in order to begin identifying substantive topics that may benefit from more integrated cross-disciplinary research on developmental plasticity. The conference focused on three primary areas of development:

- Stress and Early Development
- Cognition
- Social Behavior

Within each area, symposia were organized to capture the perspectives of

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multiple levels of analysis including molecular and cellular neuroscience, behavioral neuroscience, behavioral science, and psychiatry. In addition, newer investigators presented their work at a poster session, and roundtable seminars were convened for more in-depth discussion of key conceptual issues. The conference concluded with a series of Master Lectures on the progress and insights of several senior scientists based on their pursuits of integrated programs of research in developmental plasticity. This introduction provides an overview of the major themes discussed and highlighted during the conference.

Developing Common Frames of Reference

In his keynote address, David Kupfer proposed a challenging goal: the many disciplines involved in the study of developmental plasticity need to develop a common language by which to exchange ideas, data, and results. Over the past several decades, tremendous growth has occurred within the disciplines of neuroscience, behavioral science, and psychiatry, with each discipline being further specialized into a wide array of subdisciplines and specialty areas. Although this specialization has and continues to afford significant advances in science, it comes at the cost of narrowed awareness of methods, terms, and literatures across related disciplines. Thus, in order to address scientific questions that span disciplinary boundaries, scientists need to work at developing common conceptual and methodological languages by which to exchange ideas and data and create more integrated programs of research.

The challenge of developing a common language resounded throughout the conference. Martha McClintock, in her discussion of "Symposium I: Stress and Early Development," suggested that metaphors can be a powerful way of building a common language. She went on to generate a provocative metaphor for the conference, likening developmental plasticity to the process of a potter sculpting clay, where the environment sculpts the basic biology and provides the energy for change in the developing organism.

As with any good metaphor, this one facilitated interdisciplinary discussion of fundamental assumptions and conceptualizations of developmental plasticity. For example, to what extent do genetics constrain the process of development? To what degree can these constraints be modified by interactive and environmental events? Once modified, how amenable is development to further change?

To carry the metaphor further, how is development like the sculpting of clay and how is it like the sculpting of stone? Do different stages of brain development have different degrees of malleability? For instance, in the research discussed by Pasko Rakic on prenatal brain development and cell migration, a "sculpting stone" metaphor may be more useful in conceptualizing biological-environmental interaction, since environmental inputs during this period disrupt development with permanent consequences in the location and number of neural cells in specific regions of the brain. In contrast, the "sculpting clay" metaphor may be more appropriate when considering later phases of development, such as the research presented by Elizabeth Bates on early language development and the ability to maintain language following early brain injury.

In addition to metaphors, it was recognized that agreement on key terminology could greatly facilitate cross-disciplinary communication. Of note was repeated reference to terminology introduced by William Greenough—"experience-expectant" and "experience-dependent" brain plasticity (Greenough and Black 1992). Experience-expectant plasticity refers to structural neural development that relies on or expects appropriate experience with the environment to develop normative functioning. A classic example is the research by Wiesel and Hubel (1963) on the effects of visual occlusion on the development of the visual system in cats. Experience-dependent plasticity refers to changes in neural structure or connections that result from experience, as in many forms of learning.

These terms could be applied to many of the ideas and research findings presented at the conference. For example, Patricia Goldman-Rakic discussed her research demonstrating the incredible specificity of cells in the prefrontal cortex for the location and form of environmental stimuli. One could test the degree to which this specificity is the result of expected interactions with the environment as well as how refinements in this specificity may be brought about by or depend on forms of experience. The research described by Myron Hofer provided rich examples of both experience-expectant and experience-dependent development by showing how specific components of rat maternal care are necessary for regulating the immature biological systems of the young.

These terms are particularly useful in that, with some expansion, they could be used to describe development at various levels of functioning. For example, one could refer to experience-expectant development at the molecular and cellular level, as when neurons depend on the activity of adjacent cells to mature and refine. At the behavioral level, the term "experience-expectant" could be expanded to describe certain behaviors that cannot develop without expected forms of environmental stimulation. Thus, terms such as these have great potential for building a common language for cross-disciplinary exchange.

Bidirectional Influence and Integrated Levels of Analysis

Another point of commonality from which to build an integrated science of developmental plasticity is the idea of bidirectionality of influence. Throughout the conference, the outdated notion of "nature versus nurture" was set aside in favor of a newer, more integrated paradigm in which processes and outcomes of development are viewed as products of bidirectional interchanges between biology and environment.

Interactions with the environment affect and change the course of biological development. These changes in biology, in turn, affect behavior and functioning of the organism. Changes in behavior then alter the experiences stimulated in and provided by the environment, which in turn perpetuate further development. Thus, the traditional questions asked by a nature versus nurture paradigm such as "How much of development is the result of nature and how much is the result of nurture?" are being replaced by more sophisticated questions regarding the timing of biological-environmental interactions. When is development experience-independent, experience-expectant, or experience-dependent? How do these environmental effects vary across types and phases of development? What specific biological mechanisms and environmental experiences are involved?

In order to appreciate the full scientific impact of adopting bidirectional models, however, the array of levels of influence within both the biological and the behavioral domains must be expanded and mutually acknowledged. As pointed out by Robert Hinde, in the past, studies frequently were conducted from either a biological or behavioral perspective. Studies from a biological perspective acknowledged the importance of multiple biological levels of analysis (e.g., molecular, cellular, organs, and systems), but referred to behavior in more general and undifferentiated terms. Similarly, studies conducted from a behavioral perspective acknowledged the importance of multiple behavioral levels of analysis (e.g., individual, interaction, groups, and social-cultural), but referred to biology in less specific ways.

For advances in neuroscience to be realized and integrated with advances in the behavioral sciences and vice versa, the respective disciplines need to expand and acknowledge the complexity inherent in the other. The concept of multiple levels must be expanded to include functioning that spans from molecular and cellular processes, through individual behavioral functioning, to cultural and societal norms. Although much has been learned through analyzing various levels within both the biological and behavioral domains, the interaction between levels of functioning that span these domains is most intriguing and provides the greatest opportunities for advancing our understanding about how organisms function and adapt in a changing environment.

Using this expanded array of multiple biological and behavioral levels in combination with the concept of bidirectionality can result in the conceptual framework described by Robert Cairns (figure 1). Although this conceptualization can at first glance appear scientifically unwieldy, the complex array of possible pathways provides a logical map by which to locate where specific research conducted to date fits and, perhaps more importantly, point out yet-to-be-explored directions and questions for future research.

An example of the benefits afforded by this expanded framework can be seen in the presentation by James Leckman. He skillfully interwove research from genetics, neurobiology, endocrinology, individual behavior, and social-cultural levels of analysis to propose the role of preoccupations in establishing

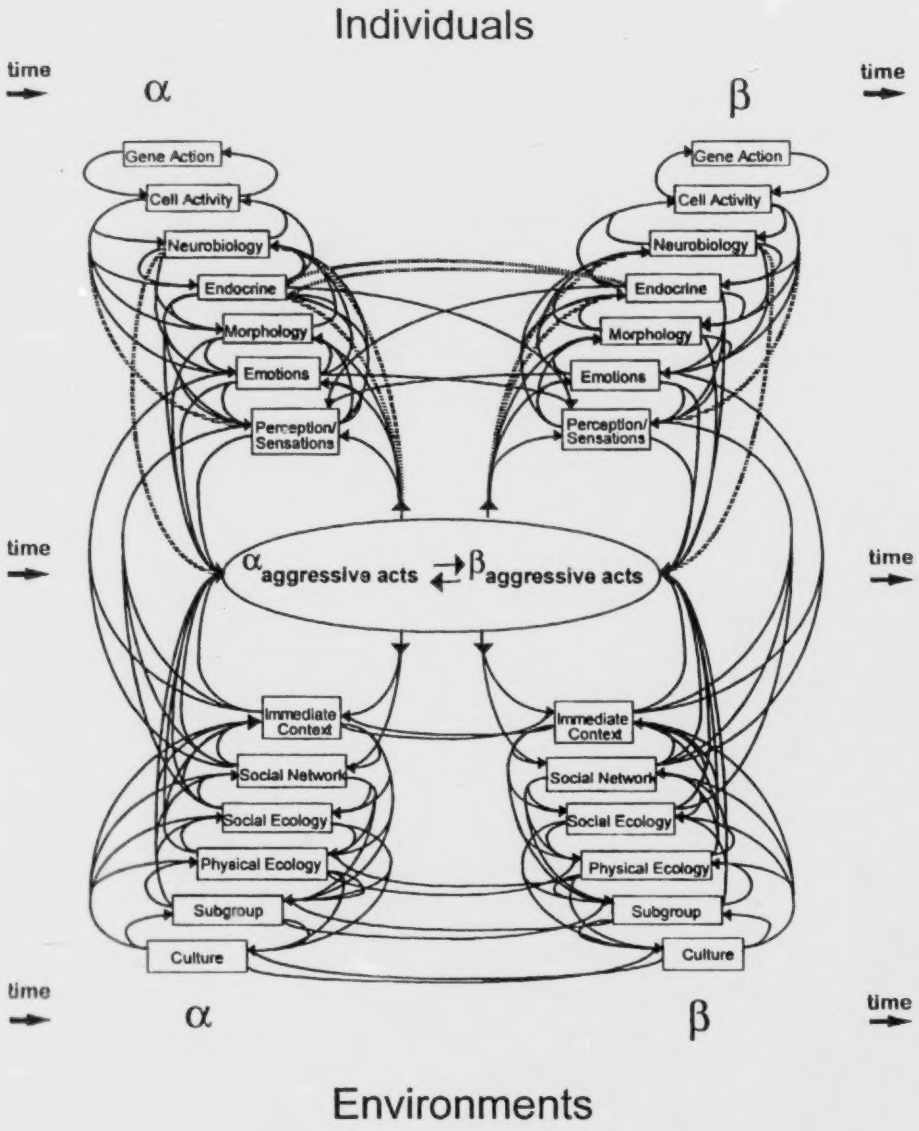

Figure 1 Schematic to describe two interacting individuals (α, β) in terms of the system of relationships that have been observed within each organism and within each environment. The solid lines show firmly established relationships between levels observed in empirical studies of aggressive behavior of mice. The dotted lines show empirical relationships that have been reported but which require further investigation. The arrows represent time and directionality of the relationships (from Cairns 1996, p. 50 © Novartis Foundation 1996).

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close social bonds (such as love) and how this perspective could be used to enlighten our understanding of obsessive-compulsive disorder.

Issues of Timing

The study of development and plasticity involves the study of change over time. Robert Emde in his discussion of research on early walkers reminded us that normative transitions in development are periods marked by change, and as such, they provide ideal windows for the study of mechanisms involved in plasticity. Although attention has been given to describing developmental transitions within individual levels of analysis, very little work has been done to address how developmental transitions within one level of analysis are arranged temporally with transitions at another level, or how transitions in one level of analysis may accelerate or impede development in other levels. For example, the research discussed by Martha Constantine-Paton on early synaptogenesis leads to questioning whether the development of inhibitory gating mechanisms within the brain influence the degree and timing of plasticity at higher levels of organization. Similarly, Eugene Emory's work on prenatal physiological and behavioral development stimulates questions about what kinds of cellular and system development are plastic during the first weeks postpartum and how the movement from intrauterine to extrauterine environments affects this development.

Addressing the timing and sequencing of development across multiple levels of functioning will shed important light on the age-old notion of critical or sensitive periods. First, the levels of analysis that constitute the sensitive period can be more specifically identified. Second, the temporal nature of the sensitive period can be more clearly specified. Last, the mechanisms involved in creating and closing the sensitive period may be determined. These fundamental issues that biological and behavioral scientists have independently been puzzling over for years may now be answered through more integrated research in developmental plasticity.

It is important to note that these issues of timing and sensitive periods are not limited to prenatal and early development. Important biological and behavioral transitions occur through out the lifespan—puberty, the transition to adulthood, childbirth and the transition to parenthood, menopause, and old age. Indeed, in order to understand the significance of the changes in functioning brought about by alterations in early developmental plasticity, one may need to study functioning later in life or with the next generation. Joseph Coyle stressed the importance of examining nongenetic transgenerational causes of social impairments where harsh or toxic environments may affect early brain development, which in turn limits the ability to meet the challenges imposed by such environments, thereby affecting the ability to raise the next generation.

Alternative Pathways

Sensitive periods in plasticity may have positive as well as negative consequences. On the positive side, these periods of development can be viewed as opportunities to assist or intervene in order to enhance adaptive development of the organism. On the negative side, these periods may be viewed as times of vulnerability when environmental experiences are not sufficient or provide inappropriate contexts for development. The plasticity that allows the organism to accommodate to less-than-optimal experiences by developing alternative pathways could threaten development in other areas of functioning or limit adaptive capacities later in development.

Examples of potential negative consequences of early plasticity were discussed by several of the conference participants. Bruce McEwen pointed out how the hypothalamic-pituitary-adrenal (HPA) axis necessarily becomes active to enable cells and organs to adapt and respond to environmental challenges. Just as importantly, however, this increased activity needs to turn off; the cost of maintaining this increased activity can result in cumulative damage and eventual neuronal atrophy.

Similarly, Dante Cicchetti discussed the behavioral and psychological costs of adapting to less favorable environments in the case of children who have experienced early maltreatment. Some of these children develop an early style of social vigilance and social control that may be adaptive in dealing with an abusive parent but maladaptive in relating to peers and other adults. Felton Earls provided another poignant example of how children growing up in socially deprived environments experience increased HPA activity that is accompanied by the behavioral cost of poorer cognitive and social developmental outcomes.

Plasticity: Hope and Challenge

Why is developmental plasticity in brain and behavior so important to mental health? Identifying the mechanisms and the timing by which these mechanisms operate could produce a greater understanding of the multiple and interconnected levels of functioning that lead to adjustment, as well as a much better understanding of when and how functioning becomes impaired. Indeed, the inability of the organism to take advantage of environmental experience during specific periods of development, that is, a lack of plasticity, may be a fundamental reason for disorders. For example, in discussing his recent research on the Fragile-X Syndrome, Allan Reiss indicated that children with this disorder show a premature plateauing in cognitive functioning and their cognitive abilities are less affected by the social-familial environment compared to normal functioning children. This suggests that the genetic Fragile-X abnormality may limit certain forms of neural plasticity, thereby preventing the organism from fully seizing experience-dependent opportunities.

By examining the natural developmental timing of sensitive periods for plasticity, scientists will have firmer data to inform decisions about when to implement prevention (before or during the sensitive period) or intervention (after the sensitive period). As was discussed by Albert Galaburda in regard to language disorders, the neural and environmental mechanisms responsible for developing disorder may not be the same mechanisms or systems involved in maintaining the disorder. Similarly, the research discussed by Helen Neville shows that within the first 4 years of life, the visual and auditory regions of the brain can respond to similar stimuli. However, when one system, the auditory system, is not functioning optimally, the other system, the visual system, will acquire more territory in the brain. Thus, the neural pathways, processes, and influential environmental experiences could be quite different before than after adaptation, which in turn will affect the targets and strategies used in prevention and intervention.

Overall, the study of developmental plasticity will have tremendous impact on how we understand the contributions, interactions, and mechanisms of biology and environment in brain development. Gaining knowledge about these mechanisms and their pattern of timing and influence across multiple levels of analysis will be challenging and complex. Yet, the potential for reward, in terms of identifying not only the windows of opportunity for change but also the mechanisms of change, is profoundly inspirational and drives forward the scientific enthusiasm for pursuing integrated cross-disciplinary research in developmental plasticity.

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Keynote Address

Developmental Plasticity: Is It the "Plastics" of the 90s?

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In this chapter, we argue in favor of the conference's major goal, namely, that cross-disciplinary research accelerate conceptual integration of research in developmental plasticity, particularly in stress and development, cognition, and social behavior. However, achievement of such goals necessitates a common language across disciplines and a vocabulary that places investigators on the same playing field regardless of specific disciplines.

As a term, "plasticity" has many definitions, and its own diversity of meaning may either lead us to a Tower of Babel or provide a platform for creating guidelines for scientific conversations and collaborations. This brief overview discusses several definitions of plasticity, reflecting specific disciplines or areas of research, some of the steps needed to move toward more integrative research, and several efforts to illustrate the successful translation of "plasticity" from basic neuroscience to more clinical research ventures.

Initially, for this type of interdisciplinary research, we need to define the most important questions that can be addressed with this approach. Factors related to critical or sensitive periods, coupling or noncoupling of the central nervous system (CNS) changes and environmental "events", and readiness should exemplify the dialog among researchers. A framework for these activities is proposed that specifies four phases: language and communication, conceptual models and methodology, strategies for implementation, and policy implications.

As an example of an ongoing experiment, we discuss the conceptual framework developed within a MacArthur Foundation-sponsored Network on Psy-

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chopathology and Development for activities focused on the role of plasticity in the development of psychopathology. It highlights the key dilemmas raised in attempting integrated research on developmental plasticity.

Definitional Diversity

One of the most important initial issues is to agree on definitions of plasticity and perhaps utilize a hierarchial framework. First, it is useful to make a distinction between behavioral and neural plasticity. Behavioral plasticity can be thought of as a manifestation or expression of neural plasticity (e.g., the neural mechanism that shows recovery of function). One approach in defining plasticity across all levels would be to use the hierarchy in the brain as defined by Floyd Bloom (1995) and assume four levels of neural plasticity: (1) the molecular level concerned with receptors and channels, (2) the cellular level concerned with specific neurons, (3) the system or multicellular level, and (4) the behavioral level.

Various definitions of neuroplasticity may not always distinguish between possible alterations in function at particular levels in the system, such as at the synaptic cellular level, different areas in the brain, and different systems. Indeed, Shaw and colleagues (1994) ask the basic question: "Is the underlying nature of the neural modification reducible to similar processes at different levels within the same system, the same for different neural systems, the same in one system at different stages in development, or the same in one system under different experimental and natural conditions?"

A third way of describing plasticity was generated by Greenough and colleagues (1992) using the concepts: (1) experience-independent development consisting of both activity-independent and activity-dependent system development, (2) experience-expectant development, and (3) experience-dependent development. While experience-dependent development may be the most useful concept for thinking about psychopathological or pathological developments, we should not neglect experience-expectant development as a potential contributor to psychopathology. For example, if experience-expectant development is defined as development in response to (species-dependent) usual input from the environment, one could consider attachment behavior as reflecting an experience-expectant process; if minimal conditions are not met (e.g., by having a neglectful or abusive caretaker), pathology can result.

Greenough's interest in learning and neural plasticity has fostered considerable research based on this framework. As one very recent example, Abraham and Bear (1996) introduced the concept of metaplasticity. They point out that activity-dependent modifications of synaptic efficacy are crucial to the storage of information in the brain. Metaplasticity embraces the notion that synaptic or cellular activity can leave a "trace" and change the ability to induce subsequent synaptic plasticity (e.g., long-term potentiation [LTP] or depression). In short, metaplasticity represents a higher order form of synaptic plasticity.

A fourth related definition concerning the plasticity of the developing brain is provided by the late Roland Ciaranello and colleagues (Morilak et al. 1995). They suggest that "the immature brain shows an amazing ability to adapt to changes in its environment and to fine tune its connections during development" (p. 676). They also point to the fact that normal development unfolds as a series of timed genetic events, the coordinated expression of which depends on appropriate environmental stimuli. Furthermore, the observation of transient expressions of important developmental factors or functions, at either the gene or cellular level, at critical times in ontogeny is crucial in providing a better understanding of developmental neurobiology.

As we can see, plasticity can truly be the concept for all seasons. In the next section, we provide different examples that can be derived from this range of definitions.

Examples of Neuroplasticity

At the most basic level, McConnell (1995) presented elegant strategies for neuronal diversity in the developing central nervous system. One example, using transplantation techniques in the developing cortical brain, indicates an intrinsic developmental clock. Interaction between environmental factors and timing on multipotent cortical cells leads to different phenotypes. These studies provide an indication of extended neural plasticity after cell migration into the neocortex.

Shaw and colleagues (1994) developed a model suggesting that synaptic neuroplasticity must arise from a series of interrelated molecular events resembling a cascade in which individual elements may differ radically from system to system. They describe a quantitative computer simulation of certain age-dependent stages in the receptor regulatory cascade that may interact to produce LTP-like effects. Their model may provide some insights for critical-period neuroplasticity.

Strategies developed to understand psychotropic drug action are now invoking drug-induced neural plasticity to specify the potential long-term action of drugs in the brain. Hyman and Nestler (1996) recently summarized their paradigm for the initiation and adaptation of a psychotropic drug. The adaptation process of repeated stimuli on an initial target protein can lead to stable long-term drug effects. This process is probably carried out by adaptations in postreceptor signaling pathways, including regulation of neural gene expression. Such a model can provide insights into the onset of behavior change as influenced by cellular signaling.

Another example demonstrates how social experience can modulate neural circuit function by controlling the effect of a neuromodulator on the response to a particular neuron (Yeh et al. 1996). In crayfish, the serotonin effect on the neural circuit for tailflip escape behavior depends on the crayfish's social experience. While serotonin reversibility enhanced the response to sensory stimuli of the lateral giant tailflip neuron, it reversibly inhibited the response in subordinate crayfish. Serotonin persistently enhanced the response in socially isolated crayfish. This study represents an interesting example of interaction between behavior, environment, and synaptic plasticity.

While examples of plastic organizational changes in the adult primate and nonprimate CNS are now common for somatosensory, visual, and motor systems, only recently have investigators begun to examine the effects, not of recovery of function after CNS injury, but plasticity associated with learning and changes in response to environmental demands. Elbert and colleagues (1995), using magnetic source imaging, demonstrated increased cortical representation of fingers of the left hand in string players. Not only was this cortical representation larger than that of the control group, but within the string player group, a significant correlation was found for the age at which the person had begun to play ($r=0.79$, $p<.01$) (although experience may be a confounding factor).

One final example of plasticity can be appreciated from the considerable advance recently shown by Tallal, Merzenich, and colleagues (Tallal et al. 1996; Merzenich et al. 1996). They developed and successfully implemented a new computer-game training program for language-impaired children with particular difficulty in oral language. Children with an inability to recognize the short-duration sounds of spoken speech improved significantly in 1 month using "temporal processing" techniques similar to primate research demonstrating cortical plasticity.

These examples portray the dimensions of critical-period neuroplasticity, interactions of neurotransmitters and cellular signaling, and cortical organization. The next two brief sections point out strategies to accelerate the integration of this research activity into research on brain and behavior over the early lifespan.

Strategies

The examples given indicate the richness of current research in plasticity, which now has extended to clinical applications of basic neuroscience strategies. What will be needed to move this type of work to a broader, more integrative level of activity? How can we accelerate this trend within an organized framework?

One approach would be to provide models of systematic inquiry to be supported by private and governmental organizations. Such advances will not be achieved in a short timeframe, since these efforts require a time sequence that includes at least four features (table 1). First, we need to provide an opportunity for improved language and communication across scientists from different disciplines. Second, the initiation of efforts that provide for the specification of conceptual models and the ensuing methodology to test hypotheses should be encouraged. Such activities then need to be provided the

TABLE 1. *Developmental phases*

- Language and Communication
- Conceptual Models and Methodology
- Strategies for Implementation
- Policy Implications

necessary time and resources for implementation to be conducted in concert with potential policy implications.

Such a broad effort will also necessitate a commitment to research training and mentoring at different levels. The next section provides a living example of this process occurring in a foundation-supported initiative.

MacArthur Network on Psychopathology and Development

While activities developed by the Network on Psychopathology and Development involve children 4 years of age and older, we are aware that individual entry points into pathways toward psychopathology may well occur earlier. Since we are trying to connect what we are measuring to later psychopathology, we decided to focus (at least in our human studies) on the earliest possible age at which such connections can be made in a meaningful manner. However, in selecting specific projects to undertake, we look for studies that allow us to make connections to earlier development: either by working with cohorts of children on which data from very early development onward are available or by using methods that allow us to query across species and, thus, to connect with the extensive neuroscience knowledge base on early development.

In this sense, our focus on ages 4+ certainly does not reflect the view that the brain remains entirely plastic through age 4 and, thus, amenable to interventions to prevent the development of all psychopathology (perhaps some?). Rather, it reflects our strong objective to connect what is known about earlier developmental periods and what is known about psychopathology. We are assuming that events that occur in the first few years of life, be they biological or environmental, do not result in the permanent closing of doors. These events may be stage setting, and in some domains, stage limiting (e.g., no language input for the first few years always results in disturbed language later), but in most domains a "window of opportunity" continues to exist.

While we acknowledge that early life events are very important later in life, they are not necessarily deterministic. We need to fill in the great blanks that currently exist—namely, what are the earliest signs and symptoms or precursors of psychopathology that occur at an age when they can be meaningfully measured? Many would say that with the current instrumentarium, that age limit is about 8. This leaves a tremendous gap between the extensive infancy and basic literature and the literature on developmental psychopathology or psychopathology arising during later developmental phases.

Furthermore, much of the existing literature on child development and developmental psychopathology neglects to incorporate a biological or neuroscience perspective and, thus, will not anytime soon be able to contribute much to clarifying what gets laid down very early and becomes immutable and what remains malleable into later childhood. As a matter of fact, much of this research operates under the assumption that ALL remains malleable throughout development and rejects ANY notion of "immutability." On that background, we selected our thematic foci—among them, trying to build a conceptual and methodological infrastructure that will allow us to make such much-needed connections, with the ultimate objective to place ourselves in a better position to determine where to focus effective intervention strategies and when they are best applied.

Our network has been engaged intellectually in issues of plasticity versus early determinants of psychopathology ever since its earliest planning activities. If there is something like a "bottom line" in our thinking about these issues at this point, it is the following: Plasticity can mean many things, and there is more to it than the temporal dimension, that is, there is not just more plasticity earlier in life than later. For example, one could even argue that the flexibility of thought and personality that comes with increasing age relative to the first 15-20 years of life is an example of continuing plasticity. A number of investigators have identified reversibility of functional impairment following CNS damage, suggesting a more open-ended view of development. On the other hand, they are all focused on motor domains, not social or cognitive, and it has become our view that higher level functions may well have very different critical periods and windows of opportunity and risk than motor or sensory functions.

A second major issue we have talked much about, particularly in considering plasticity as it relates to higher level functions, is that "plasticity cuts both ways" (Greenough and Black 1992). If disruption or insults (biological or environmental) occur early, it may be good on one hand in that the brain has the plasticity to rearrange itself and compensate, but bad on the other for the very same reason, namely, in that any compensatory effort opens up the possibility of a whole new set of connections in the brain and that in developing alternative connections the child will develop into something it would not otherwise have been—that is, its trajectory will be altered.

Whether that fact is good or bad depends on the outcome of interest. For example, if children, in compensating for stressors imposed early in their development, become highly competent but also quite depressed, it is good in terms of the outcome "academic competence," but it is bad in terms of the outcome "mental health" or "social competence." Furthermore, the same experience and need for compensatory connections may affect individuals differentially depending on their genetic/biological predisposition or endowment. Thus, what represents a "steeling experience" and generates resilience in one child may enhance vulnerability or "break" another child. The possible outcomes of the same experience in different children may be: (1) a competent and emotionally stable child, (2) a competent but emotionally unstable child, or (3) a child that is neither competent nor emotionally stable.

Thus, we cannot really ask what any individual trajectory would have become without adverse impact during early development. Since only a portion of brain development occurs independent of experience, and most in an either experience-dependent or experience-expectant fashion, it is extremely difficult if not impossible to determine which type of development is responsible for the specific profile of an individual's higher functions. Since both the environment and biological development are dynamic systems, and systems that unfold not independently of each other but most likely in close and constant interaction, sorting out which determines what and in which manner is a methodological and conceptual challenge.

Finally, psychopathologies are highly complex phenomena with a variety of biological and behavioral features. Their development within the highly dynamic process of individual development is a "rapidly moving target" that is exceedingly difficult to investigate. Thus, understanding plasticity issues as they relate to the development of psychopathology is no small challenge. We have neither the conceptual nor the methodological infrastructure in place to approach these important questions in a manner that gives sufficient credit to their complexity.

Thus, we may have to begin this process with imperfect tools, much as using a still camera to capture a dynamic process. However, even still pictures from various parts of a movie will give us better clues to the film's plot than no pictures at all. We feel that we are setting out to get some still pictures from a movie and we know something about the beginning—when a rather fast-moving, action-packed plot unfolds—and some information on parts much later in the action—when things appear to have settled down and become more stable. We know very little about what happens in between. Some of the characters we see in the beginning do not resemble those we see later on. Some are clearly the same, but we do not know what happened to them in the interim. But we have enough to begin to speculate. Currently, we do not have the tools to study development in real time, but we hope to take many still pictures with little temporal delay, so that we may cartoon them in such a way that we simulate development in real time.

In closing, we should remember:

> The human brain is remarkably plastic. It adapts to a variety of circumstances, forms memories of experiences, and learns procedures. It can

> become dependent on drugs or produce disabling psychopathology, and it can recover. The plasticity of our brains and, therefore, our ability to learn and adapt is at the heart of our evolutionary success in nature and of our cultural evolution as well. (Hyman and Nestler 1993, p. 95)

ACKNOWLEDGMENTS

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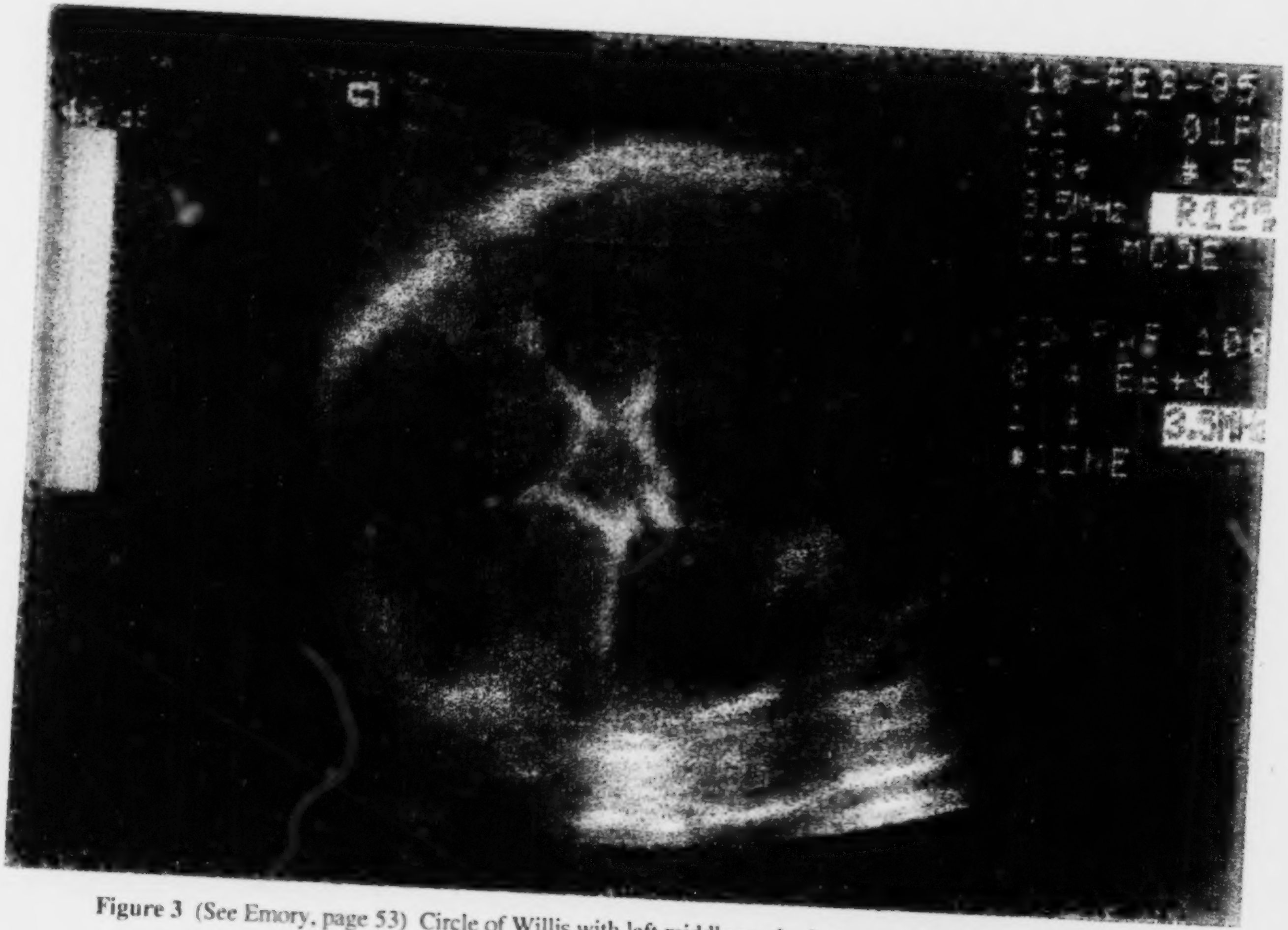

Figure 3 (See Emory, page 53) Circle of Willis with left middle cerebral artery at 6 o'clock.

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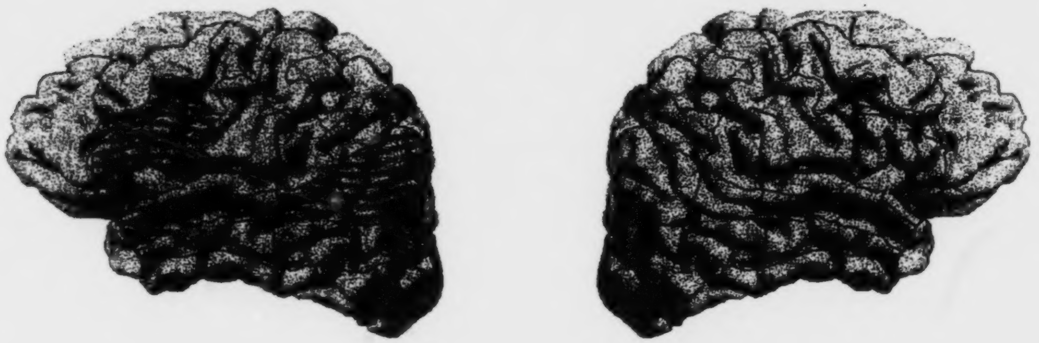
Written English -- Native Speakers



American Sign Language -- Native Signers

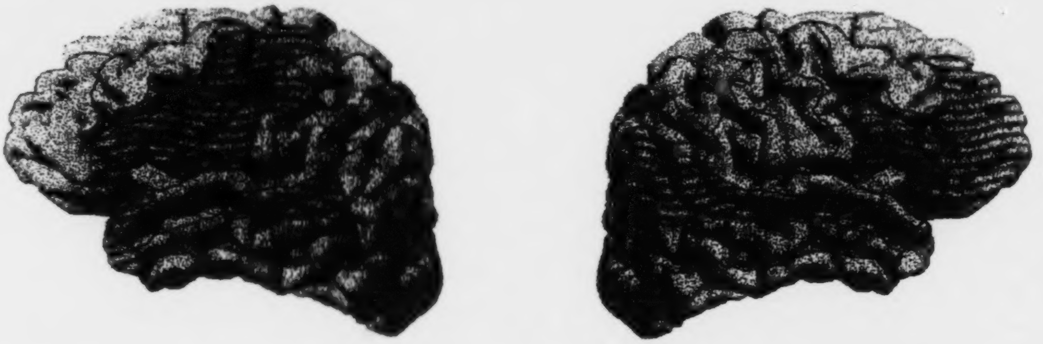


Figure 3 (See Neville, page 181) Red stripes indicate areas that display significant increases in MR signal intensity when subjects process sentences.

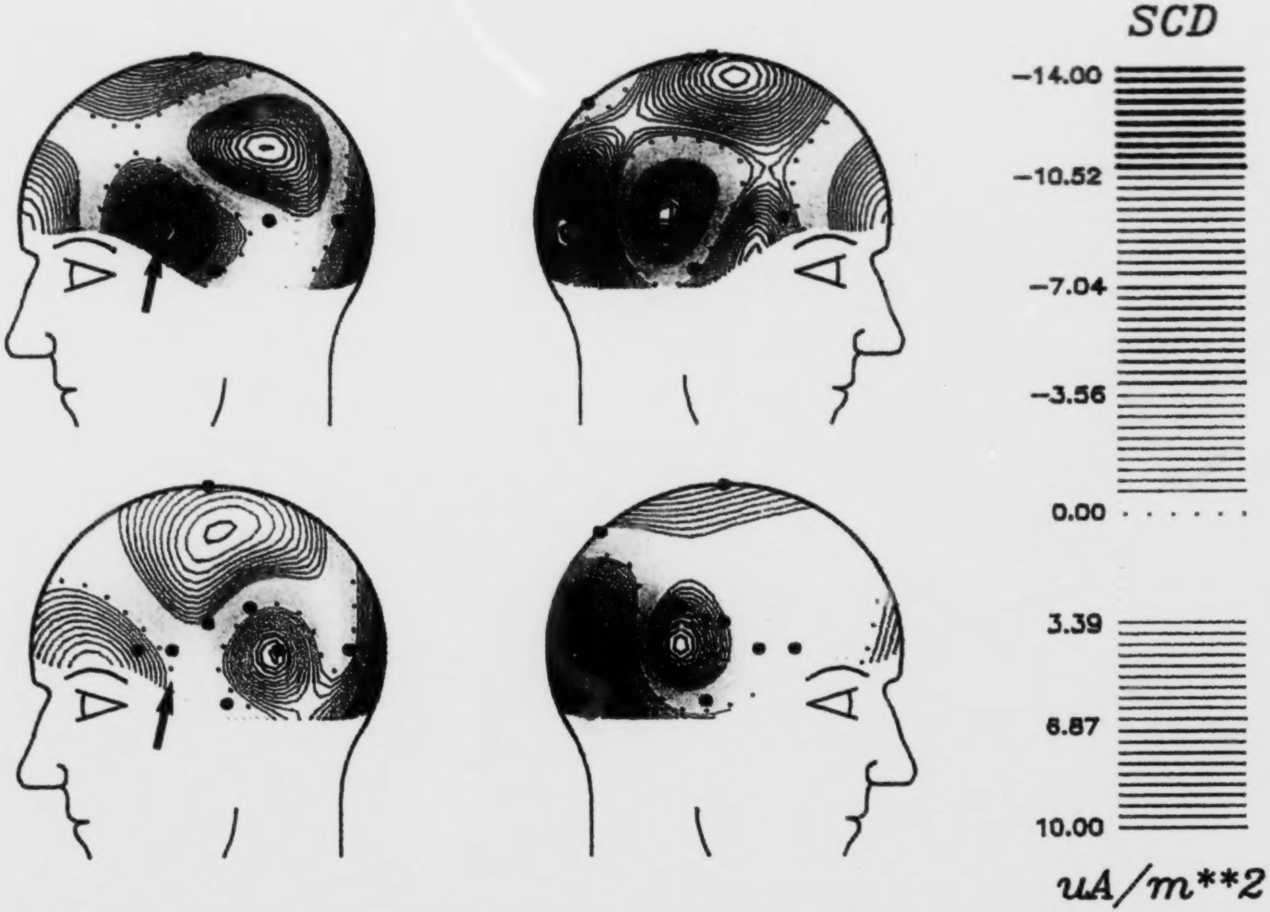


Figure 4 (See Neville, page 182) Distribution of current flow for the N280 peak elicited by closed-class words in English. Maps in the top row show the prominent response in the left hemisphere averaged across 17 hearing subjects (blue, marked by arrow). Bottom row maps display results from 10 congenitally deaf individuals who lack the response (arrow). (From Neville et al. 1992, figure 9, © Oxford University Press, used with permission.)

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Stress and Early Development

Activity-Dependent Synaptogenesis

Martha Constantine-Paton

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ABSTRACT

In much of the developing nervous system, electrical activity guides the formation of neural connections, with lasting effects on adult brain function. A range from subtle to pronounced defects in neuronal excitability and function could result from abnormal patterns of activity in the young brain. Many projections are organized by selective reinforcement of synapses that are activated simultaneously on a postsynaptic cell during a sensitive period in early life, a process that often is controlled by calcium entering through the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. The magnitude of calcium entry through this receptor depends on its subunit composition, which varies with age and brain region. Although receptor subtypes that admit large calcium currents are permissive of synaptic plasticity, they also increase neural vulnerability to excitotoxic cell death, which is linked to elevated intracellular calcium concentration as well.

In most regions of developing brain, activity levels increase with maturation. Thus, it is adaptive for NMDA receptor subtypes that admit large calcium currents to be prominent in the brain during early periods of synaptic organization when activity levels are low and to be replaced in older animals by NMDA receptor subtypes that admit less calcium as synapses become more active and more effective. However, a side-effect of this NMDA receptor downregulation would be decreased synaptic plasticity. We propose that this transition from NMDA receptors with high calcium permeability to less permeable receptor subtypes is itself activity dependent, resulting in a feedback mechanism that maintains calcium

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entry through NMDA receptors within some optimal range as activity levels vary throughout development.

This chapter outlines recent work in our laboratory aimed at understanding the interaction between early activity patterns, the regulation of NMDA receptor expression and function, and the structural and biochemical consequences of this interaction. The work is concentrated on the retinotopically organized visual layers of the rat superior colliculus where the patterning of the inputs is well described and easily assayed. Moreover, neurotransmitter receptor activation in this region can be reproducibly perturbed by chronic receptor agonist and antagonist treatments, and the visual layers of the colliculus are discrete and easy to isolate for analyses of receptor subunit transcripts and proteins associated with synaptic transmission.

The field of developmental neurobiology is receiving increasing attention as we progress through the nineties. It is a field with breathtaking potential for understanding how brain plasticity is regulated, how environment shapes our lives, and how slowly evolving brain dysfunction can pervert the natural processes of circuit development. However, developmental neurobiology is also a field that spans all biological levels from genes to behavior and virtually all of the techniques presently available to life scientists. In this era of limited resources and conservative review panels, neural development is becoming an increasingly fractionated discipline. Thus, a symposium like this that seeks to build bridges specifically between those areas of developmental neurobiology that most directly affect behavior and those areas of behavioral biology that are most affected by development is most welcome.

The subject of this chapter constitutes one of these areas. We work on synapse formation in the central nervous system (CNS). Synaptogenesis is fundamental to the organization of neural circuits and to the subsequent behavior that these circuits control or modulate. The prevalence of genetic or environmental factors in this process of circuit formation has been debated for over a century (Lehrman 1970). Thirty years ago, synapse formation was viewed as a highly determined series of wiring events, which in some instances, could be fine-tuned by "experience" or "functionally validated" during brief developmental windows (Hamburger 1970; Jacobson 1970).

Current work in synaptogenesis reflects a revolution in the thinking of brain scientists, because the dichotomy between genes and environment is disappearing. We now recognize that activity that is "spontaneous" and mediated by local contacts within the developing brain of the fetus (Maffei and Galli-Resta 1990; Meister et al. 1991), in addition to activity reflecting the numerous experiences of childhood, have a formative impact on the patterning and strengths of the connections that develop over a prolonged period of brain maturation. The wiring of cell group to cell group may be orchestrated by complex genetic programs, but once synapses are formed, activity and environment control the genes that are expressed, and these in turn can change brain structure and brain function (Laufer and Changeux 1989).

It follows that abnormal early activity patterns, by disrupting normal synaptogenesis, can have potentially disruptive effects on brain function and behavior and that the brain is susceptible to such effects over much longer developmental periods then had previously been suspected. Stress produced by prenatal or perinatal events is an important factor among a growing number known to alter early activity patterns. In addition, neuronal excitability or neurotransmission, genetic or epigenetic influences that disrupt neuron migration, maternal drug use, malnutrition, pain induced by physical abuse, and sensory or emotional deprivation will all perturb early activity patterns and affect synapse development.

Moreover, it is likely that many of these factors have lasting and sometimes pervasive effects on subsequent cognition, emotion, and behavior, only because they disturb the normal process of synaptogenesis. This last point is frequently lost in descriptions of the complexities of neurodevelopment, but it should be emphasized and recognized as a basis for hope. As I will attempt to illustrate, developmental neurobiology is beginning to understand some of the major principles of CNS synaptogenesis. Thus, it may not be long before practitioners are able to intervene for children at risk to ameliorate or mitigate the abnormal patterns of excitation and inhibition before behavior or cognitive state is irreversibly affected. In short, the fact that plasticity is inherent in synaptogenesis and that synaptogenesis is ongoing offers the hope of using interventions based upon biological mechanisms to change the brains and lives of the millions of individuals suffering mental disease or disabilities from the effects of aberrant early brain function.

Work in my own laboratory is concerned with the cellular mechanisms underlying activity-dependent synaptic change. Many of our studies use the visual layers of the superior colliculus, a region that is relatively uninteresting in terms of cognitive or emotional development. Nevertheless, concentration on this area has some distinct advantages when attempting to establish cause and effect relationships between synaptic activity and circuit development. In the rat, we know a great deal about the normal development of the superior colliculus. We know when its major inputs, those from the retina and from the primary visual cortex, enter and refine their synapses. We know when these inputs lose the ability to adjust to surgical perturbations and when they become responsive to stimuli from the outside world. We are also able to reproducibly manipulate the activity of the early synapses of the superficial layers of the colliculus by locally applying transmitter antagonists or agonists in a slow-release plastic placed on the dorsal midbrain surface.

This chapter illustrates two important points. First that synaptogenesis is a process involving several distinct stages. It is not a singular event. Second, that the outcome of the process of synaptogenesis is dependent from its earliest inception on ongoing activity. However, similar alterations in activity can have very different effects on subsequent development depending upon the stage of synaptogenesis during which they occur.

Glutamate Receptors and Mechanisms of Plasticity

Glutamate is the major excitatory transmitter in the brain, yet only within the last decade have we finally begun to understand the various receptors through which glutamate exerts its effects on vertebrate neurons. Glutamate receptors can be divided into two major classes—the slowly acting g-protein coupled receptors and the multimeric ionotropic receptors that mediate most fast synaptic events by opening an ion pore when the ligand glutamate or aspartate is bound (Hollman and Heineman 1994). Our experiments are concerned with the receptors of the latter class.

The ionotropic receptors are further subdivided into at least two families depending upon (1) the highest affinity exogenous agonists and (2) whether ligand binding is sufficient to cause current flow and a change in postsynaptic transmembrane potential. The glutamate receptors that bind the agonists α-amino-3-hydroxy-5-methylisoxazole-proprionic acid or kainate (AMPA/ KA) will pass Na^+ and K^+ ions and depolarize the postsynaptic membrane.

However, in order to function, the ionotropic glutamate receptors that bind the agonist N-methyl-D-aspartate (NMDA) have two requirements. To pass current, these receptors must bind glutamate and sit on a membrane that is depolarized from its resting state to about -40 mV. NMDA receptors have a binding site for magnesium in their pore. Ligand binding will open the pore, but simultaneous membrane depolarization is necessary to eject magnesium and allow other ions to flow (Mayer et al. 1987). The active NMDA receptor channel, like the AMPA/KA receptor channel, passes Na^+ and K^+ ions. However, unlike AMPA/KA receptors, NMDA receptors also pass significant amounts of Ca^{++} (Ascher and Nowak 1988; Mayer et al. 1987).

The Ca^{++} permeability of NMDA receptors endows them with the ability to directly trigger a variety of second-messenger cascades within postsynaptic cells. Changes in intracellular free Ca^{++} are the bases of a variety of the important cellular functions such as cytoskeletal polymerization, protease activation, long-term potentiation of synaptic efficacy, gene transcription, and protein phosphorylation. Thus, ligand and voltage gating of postsynaptic Ca^{++} makes the NMDA receptor a prime candidate as a depolarization-dependent transducer of glutamate signals into critically important functions such as growth and synapse strengthening during development and of learning and memory extending into the mature brain.

However, uncontrolled levels of intracellular free Ca^{++} can also disrupt many cellular functions necessary for normal metabolism. High cytoplasmic Ca^{++} concentrations are also frequently associated with neuronal cell death (Choi and Rothman 1990). Thus, the Ca^{++} permeability of NMDA receptors also requires tight feedback control of their function. This is particularly true of the developing brain, where the growth modulating functions of Ca^{++} are in high demand and where many of the glial buffering systems of the mature brain have not yet differentiated.

Much recent work, including our own, suggests that the NMDA receptor is the major excitatory neurotransmitter receptor at young synapses and that NMDA receptor function is downregulated as development proceeds (Tsumoto et al. 1987; Fox et al. 1989; Ziskind-Conhaim 1990; Loturco et al. 1991; Siviy et al. 1991; Carmignoto and Vicini 1992; Hestrin 1992; Ramoa and McCormick 1994; Durand et al. 1996; Shi et al. 1997). A growing literature indicates that NMDA receptors both in vivo and in tissue-cultured neurons can be regulated by their activity (Fox et al. 1991; Carmignoto and Vicini 1992; Williams et al. 1992; Bessho et al. 1994; Aamondt and Constantine-Paton 1995; Resnick et al. 1995; Vezzani et al. 1995; Hickmott and Constantine-Paton 1997). Our recent work suggests that some of these interactions are circular: early function of these receptors can alter the subsequent differentiation of the circuits in which they reside, and abnormal function of the circuitry can alter the molecular makeup and function of the NMDA receptors. Thus, multilevel developmental control of NMDA receptor function may underlie many aspects of early brain plasticity.

Many studies have documented the lability of young synapses. Depending on the brain region and species, a relatively stereotyped period of peak synaptic number is followed by a falloff to mature values. This is frequently associated with a striking refinement of the long axon projections. The details of this synaptic competition and refinement mechanism are still debated, but the phenomenon is clear: inputs that are correlated with a significant depolarization of their target remain, and inputs that may be just as active on average but are poorly correlated with target cell depolarization are withdrawn. The observations seem to follow Hebb's postulate requiring coincident presynaptic and postsynaptic excitation to maintain a contact (Hebb 1949). During development, one of the most direct ways for synaptic activity to be correlated with target cell activation is for the afferents with temporally synchronized activity to converge on the same postsynaptic cells (Stent 1973; Constantine-Paton et al. 1990). This raises the question of how young inputs are synchronized in the first place.

There is now evidence for at least auditory and visual pathways. Even when activity is driven by brownian motion in the cochlea (Sanes and Constantine-Paton 1983) or intraretinal circuitry before photoreceptors differentiate (Maffei and Galli-Resta 1990; Meister et al. 1991), inputs arising from neighboring positions in the sensory periphery have highly correlated patterns of activity. Thus, this "spontaneous" activity, present in fetuses and neonates as a result of early differentiated local circuitry and adjacent positions of sensory neurons, carries information about presynaptic proximity or similarity in function of the afferent axons. These properties are encoded as the degree of correlation in the temporal pattern of afferent action potentials, and the afferents transmit this information to their synaptic terminals in the CNS where it can be used to sort synaptic terminals.

Consistent with this idea is evidence that disrupting nearest-neighbor activity patterns in the inner ear and in the retina disrupts auditory tuning (Sanes and Constantine-Paton 1983), the refinement of visual maps (Cook 1988), and retina-specific or function-specific segregation in the respective brain pathways (Reh and Constantine-Paton 1985; Dubin et al. 1986; Stryker and Strickland 1984). There is also evidence that the registration of converging sensory maps within the brain depends on a selection for correlated inputs (Feldman et al. 1996). Correlated firing has also been directly demonstrated to be involved in patterning the intracortical projections in visual cortex (Lowel and Singer 1992).

A compelling hypothesis combines the observations on NMDA receptor function and their regulation during development with the data on the existence of correlation detection during development, namely, that the NMDA receptor is the correlation detector on young neurons. Specifically, the greater the temporal association of activity in young afferents that converge on a common target, the greater the amount of current flow through the NMDA receptor, because temporal summation of the individual synaptic currents mediated by AMPA/KA receptors would relieve the Mg^{++} block of the NMDA receptor channel. The mechanism is believed to mediate competition among young synapses, because Ca^{++} that passes through the NMDA receptor as a result of cooperation among correlated inputs would trigger or facilitate a retrograde signaling system that stabilizes those inputs and in some way destabilizes poorly correlated inputs to the same cell (Constantine-Paton et al. 1990). A hypothesis very similar to this has been proposed and largely supported as an explanation for long-term changes in synaptic efficacy that take place in relatively mature hippocampus and probably underlie spatial learning (Bourne and Nicoll 1993; Constantine-Paton and Cline 1998).

In many brain pathways farther removed from the sensory periphery, experimental demonstrations that this same competitive correlation detection mechanism operates during synapse development are lacking, because there is no way to predictably and locally perturb the activity patterns within these regions and test for specific changes in connectivity. However, there is ample evidence for input competition among many of these higher brain regions (for example, see Neville this volume). In addition, the numerous well-documented reciprocal circuits within the brains of all vertebrates, as well as the high frequency with which inputs map topographically and the frequency with which inputs with similar stimulus specificities from different sources converge on higher order neurons, all suggest that a mechanism based on reinforcement of temporally correlated inputs exists at all levels.

Early Development of the Superior Colliculus

Rats are born with their eyes closed, and they do not open them until postnatal day 14 (P14). Nevertheless, the major organizational step in the rat visual pathway development takes place in this early period preceding pattern vision. Retinal ganglion cell axons are present in the superficial layers of the rat superior colliculus at birth, but they show no retinotopic organization. During the first 11 postnatal days, the axons initially arborize widely, then withdraw branches from topographically inappropriate zones, retaining only those arbors in positions appropriate to the retinal locale of their ganglion cell bodies (Simon and O'Leary 1992). During this 11-day period, synapse number increases in the superficial collicular neuropil, and both transcripts and protein for the NMDA receptor subunit NMDAR1 (NR1) increase significantly (Hofer unpublished).

In order to pass physiological levels of current, however, the NMDA receptor must be composed of the NR1 subunit and at least one other subunit of the NMDAR2 (NR2) subunit family (Meguro et al. 1992; Monyer et al. 1992). During the first postnatal week, the prominent NR2 subunit is NR2B, but this subunit gradually disappears as the neuropil matures. In contrast, levels of NR2A, virtually undetectable in the perinatal period, rise rapidly at the beginning of the second postnatal week. Thus, nearly coincident with retinotopic map refinement is a change in the molecular structure of the NMDA receptor in the collicular neuropil (Shi et al. 1997).

We have recently found that at very nearly the same time that these changes in NMDA receptor subunit composition are detected with RNase protection assays and quantitative Western blotting, whole cell patch clamp recordings from superficial collicular neurons in tissue slices reveal a precipitous drop in the contribution of NMDA receptors to the spontaneous excitatory currents (sEPSCs) (Shi et al. 1997). Interestingly, a number of earlier studies have documented a loss of structural plasticity in the retinocollicular projection at roughly the same time (Lund and Lund 1976; Mustari and Lund 1976). Transcripts for at least one of the AMPA receptor subunits (GLUR2) show a relatively constant level of expression during this early postnatal period, possibly reflecting the large number of nonsynaptic AMPA receptors that have been described.

Blocking NMDA Receptors in Early Development

The developmental correlation between levels of NMDA receptor expression, its function, its molecular makeup, and retinocollicular map refinement suggests that high levels of NMDA receptor current are necessary to sort visual synapses on the basis of retinal locale and therefore, presumably, on the basis of temporal correlations in their activity patterns. After map refinement occurs and well-correlated inputs converge on the collicular neurons, a decrease in function, probably dependent upon the changing pattern of NMDA receptor gene expression, would be an effective way to protect collicular neurons from excessive Ca^{++} influx. The same change may curtail further structural plasticity in the retinal projection.

To begin to test the hypothesis that the NMDA receptor is necessary for the withdrawal of topographically misplaced early retinal ganglion cell arbors, we placed slabs of slow-release plastic infiltrated with the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP5) on the superior colliculus of P0 pups. We found that many of the topographically inappropriate retinal ganglion cell arbors were retained in the AP5-treated colliculus (Simon et al. 1992). However, this early disruption of NMDA receptor function also stalled the differentiation of the retinocollicular synapses. The upregulation of NR1 levels failed to occur, the fine structure of the neuropil looked qualitatively immature (Scheetz personal communication), and the normal developmental differentiation of the major synaptic protein, calcium calmodulin-dependent kinase, CAMKinase II, was maintained at P0 levels in rats examined at P19 (Scheetz et al. 1996). Thus, these studies indicate that early activation of the NMDA receptor, possibly because it is the predominant excitatory neurotransmitter receptor at young synapses, or possibly because it is a major means of Ca^{++} entry into young cells (Scheetz and Constantine-Paton 1994), is necessary for the normal upregulation and differentiation of synaptic function.

Subsequent work has indicated that the period in which synapse differentiation is generally inhibited by NMDA receptor antagonism is brief. The same AP5 treatments begun at P8, when NR1 upregulation has begun, have no effect on the expression of any of the NMDA receptor transcripts examined to date (Aamondt and Constantine-Paton 1995). At the protein level, all NMDA receptor subunits are actually higher than normal. If the structural refinement of retinocollicular afferents is affected at all, the changes are not detectable with the labeling techniques that pick up grossly mispositioned retinal terminal arbors (M. Colonese personal communication).

Overactivation of NMDA Receptors During Development

The normal developmental downregulation of NMDA receptor function in the visual cortex can be blocked when normal increases in visual function are blocked by dark rearing (Fox et al. 1991; Carmignoto and Vicini 1992). This raises the possibility that the high level of NMDA receptor function resulting from the establishment of a refined map is the factor responsible for the developmental switch in NR2 subunits and for the functional decrease in NMDA receptor current at synapses in the superficial colliculus. Consequently, we attempted to drive NMDA receptors at unusually high levels prior to the completion of map refinement by treating the colliculus chronically with low levels of NMDA itself beginning at P8. Instead of the expected early drop in NR2B transcript levels, this agonist treatment resulted in an abnormally high and prolonged expression of this subunit gene. By contrast, NR1 levels and levels of the NR2A subunit were regulated normally in the treated colliculi, and levels of NMDA receptor subunit protein were not significantly altered.

A clue to what happens in this neuropil with our chronic agonist treatment was obtained when we refocused our effort to look at the development of inhibitory gamma-amino butyric acid (GABA) neurotransmission in the colliculus. Unlike the excitatory glutamatergic system, inhibitory, $GABA_A$ receptor-mediated neurotransmission is not functionally pronounced until several days after eye opening (approximately P17) in the superficial collicular layers. In normal animals, immunocytochemical staining and quantitative Western blotting for the rate-limiting enzyme in GABA synthesis glutamic acid decarboxylase (GAD) also fail to show significant levels of this enzyme until this relatively later period (Shi et al. 1997). However, in animals chronically treated with NMDA from P8, GAD levels are already high at P12. In addition, preliminary patch clamp data from similarly treated colliculi suggest that functional inhibition has matured early in response to the agonist treatment. Compared to sham treated colliculi, bicuculline sensitive currents are significantly larger in the neurons of NMDA treated colliculi, and the frequency of spontaneous excitatory synaptic currents is significantly reduced.

These data suggest the existence of an unexpected feedback control system in the maturation of neurotransmission. They support the following cascade of effects. First, the exogenous NMDA applied at P8 transiently increases NMDA receptor activity. Early overactivation of NMDA receptors either directly or indirectly acts on GABAergic inhibitory neurotransmission to expedite its maturation.

A mechanism that associates the rate of maturation of the inhibitory system with the amount of excitation in the neuropil would be adaptive, because it represents an effective control on the development of seizure-like activity. However, ongoing patch-clamp studies suggest that this early overactivation of inhibition is associated with a failure to normally downregulate the NMDA receptor.

In other words, our initial hypothesis that NMDA receptor function is normally downregulated by activity seems to be supported: inhibition is masking the normal developmental increase in the effectiveness of collicular afferents and producing an abnormal prolongation of highly effective NMDA receptors. This scenario implies at least two molecular responses to early overactivation that function to compensate each other and probably, at the circuit level, permit near normal function despite very different underlying levels of neurotransmitter and neurotransmitter receptor expression.

Building a Bridge to Behavior

The continuous presence of exogenous glutamatergic agonist and antagonist is an obviously abnormal milieu for CNS circuit differentiation. Nevertheless, it is relatively easy to extrapolate the ways in which the plasticity of both the glutamatergic and the GABAnergic systems revealed by these treatments could function during normal brain differentiation to buffer the development of a CNS region against perturbations in normal activity patterns that might otherwise cause inappropriate excitability, abnormal wiring, and/or neuronal death. Unfortunately, it is also relatively easy to envision the complex cascade of effects on circuit differentiation that could occur in higher brain regions as a result of prolonged exposure of fetuses or young children to aberrant environments. For example, prenatal exposure to cocaine has been shown to disrupt the migration of cortical neurons in monkeys (see Rakic this volume). Such ectopic neurons are likely to be exempt from the normal circuit control of their activity and, therefore, their functional output to target regions will be aberrant and potentially deleterious to normal activity-dependent differentiation in those regions.

High cortisol levels that interfere with the function of NMDA receptors during early childhood (McEwen this volume) could also be permanently deleterious to normal circuit development by disrupting the feedback control systems that appear to operate through this receptor and presumably maintain developmental plasticity within an adaptive range. Finally, numerous subtle genetic defects or polymorphisms that might singly or in association with epigenetic factors interfere with the normal developmental timing of excitation and inhibition onset in the brain could directly cause brain dysfunction or a predisposition to mental disease later in life.

However, there is also cause for optimism in this recent suggestion of aberrant excitation leading to precociously maturing inhibition. Inhibition, quite apart from its role in suppressing the responsiveness of mature neurons, may be an additional way in which the maturing brain shuts down the enormous plasticity exhibited by young children. Furthermore, the normal operation of this mechanism could mean that brain circuits may function "normally," that is, produce "normal" behavior, even though they might actually support very different absolute levels of excitation and inhibition. This indeed would be the expected result if the control of inhibition by early excitation proves to be a general property of the developing brain and if individual variations in patterns of activity exist in those circuits when they are forming and adjusting their inhibition to their excitation.

This newly envisioned role for inhibition leads to a speculation directed specifically at behavioral scientists working on the development of deprived or disabled children. There may be far more adaptive structural or functional plasticity latent in brains, at least relatively young brains, than can normally be demonstrated. This plasticity may not normally be active, because the Ca^{++} channels that allow it to occur are clamped by an abnormally pronounced inhibitory system. If one could briefly alleviate that inhibition, by training or reinforcement or by the selective use of mildly disinhibiting drugs, then perhaps there is a potential for much more plasticity and much greater recovery from early- onset disability than we would ever before have guessed.

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Hormones as Regulators of Brain Development

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ABSTRACT

Individual differences in brain function and behavior are shaped, in part, by the effects of early experience and by hormones. For example, the presence or absence of testosterone during fetal and neonatal life causes male and female brains to differ in subtle ways, both in structure and connectivity and in different responses to hormonal signals. Likewise, prenatal stressful experiences and the opposite effects of postnatal handling appear to involve adrenal and thyroid hormone actions, respectively, that program the brain to have either higher or lower reactivity to novel experiences later in life. According to this model, the subsequent actions of adrenal steroids in adult life determine the rate of brain and body aging. The hippocampal formation of the brain turns out to be one of the most vulnerable and plastic brain regions in which these processes can be studied.

The hippocampus is also a target brain area for the actions of hormones of the steroid/thyroid hormone family, which traditionally have been thought to work by regulating gene expression. "Genomic" actions of steroid hormones involve intracellular receptors, whereas "nongenomic" effects of steroids involve putative cell surface receptors. Although this distinction is valid, it does not go far enough in addressing the variety of mechanisms that steroid hormones use to produce their effects on cells. This is because cell surface receptors may signal changes in gene expression, while genomic actions sometimes affect neuronal excitability, often doing so quite rapidly. Moreover, steroid hormones and neurotransmitters may operate together to produce effects, and sometimes these effects involve collaborations between groups of neurons. For example, a number of steroid actions in the hippocampus involve the coparticipation of

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excitatory amino acids. These interactions are evident for the regulation of synaptogenesis by estradiol in the CA1 pyramidal neurons of hippocampus and for the induction of dendritic atrophy of CA3 neurons by repeated stress as well as by glucocorticoid injections. In addition, neurogenesis in the adult and developing dentate gyrus is "contained" by adrenal steroids as well as by excitatory amino acids. In each of these examples, NMDA receptors are involved. Thus, neurotransmission interacts with the endocrine system to regulate the structure and function of both developing and adult brain cells.

Development is a life-long process in which early events can have a long-lasting or even permanent impact on the capabilities of the organism (McEwen 1995). Sexual differentiation of the body and brain is a prime example, in which the phenotypic sex of a bird or mammal is determined by secretions of the gonads during critical or sensitive periods early in life (Goy and McEwen 1980; Goy 1970). Genes primarily determine the sex of the gonad, and then hormones do the rest.

In addition to the group differences of being male or female, individual differences in the brain and body are influenced by early experiences, acting in part via hormone secretion. Thyroid hormone and adrenal steroids play a role in addition to sex hormones, along with other factors that remain to be determined (Gould et al. 1991). The effects of early stressful events or of handling of newborn rat pups produce life-long differences in the reactivity of the stress hormone axis that affect the rate of brain aging (Meaney et al. 1988), and these effects are mediated in part by adrenal steroids and by thyroid hormone. This chapter provides an overview of these interactions, following an introductory discussion of the cellular mechanism of hormone action and the hormonal control of gene expression. The article concludes with an overview of the relationships between adaptation to stressful life events and the occurrence of disease.

Receptors and the Regulation of Gene Expression and Nongenomic Events

The idea that intracellular receptors that bind circulating hormones regulate the expression of genes by either increasing or decreasing the transcription of messenger RNAs began a revolution that has continued until today (McEwen 1995). We know that many kinds of DNA-binding proteins regulate gene expression. The original model showed that a steroid receptor bound to a response element in the promoter region of regulated genes increases or decreases the expression of the messenger RNA for those genes through this binding site. We now know that there are proteins which bind to other response elements and are regulated by other events, such as phosphorylation through second-messenger systems (figure 1). Moreover, if that were not complicated

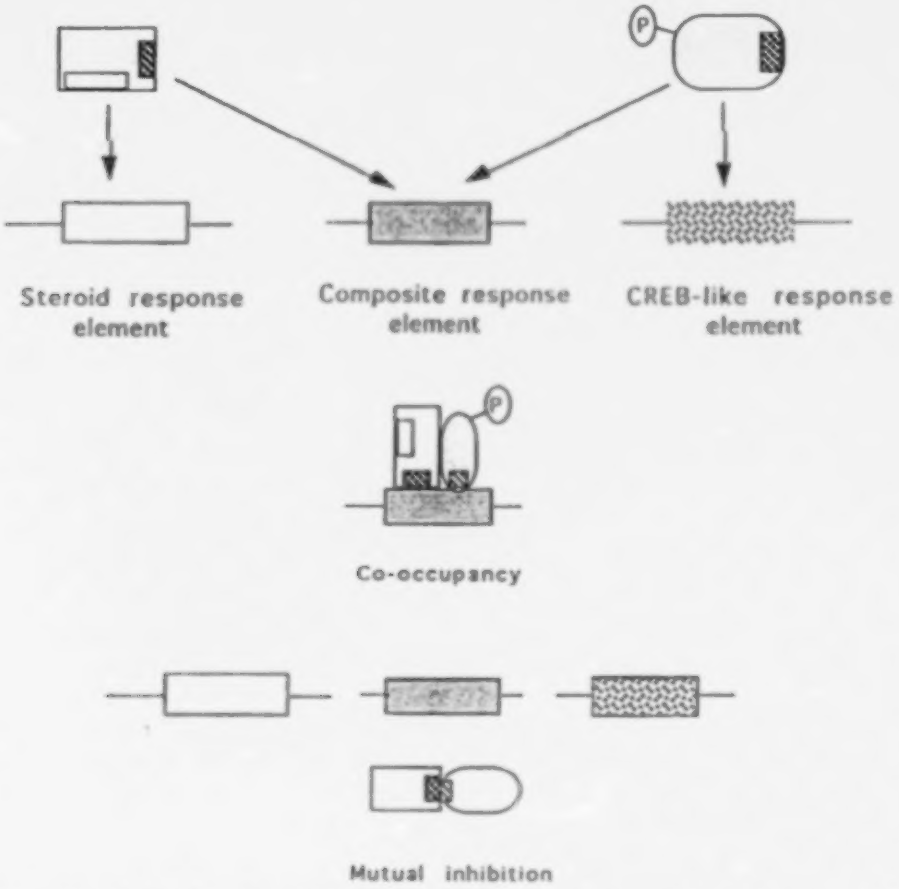

Figure 1 Steroid hormones and CREB proteins affect gene expression via separate and common response elements in the promotor region of certain genes. Besides cooccupancy of composite response elements, steroid receptors and other DNA binding proteins, like fos-jun dimers, are known to bind to each other with such high affinity that they reduce occupancy of DNA response elements.

enough, we now have examples of composite response elements which require both steroid receptors and a member of another family such as CREB or an immediate early gene, for example, a fos/jun heterodimer, and the combination of these two proteins is required for regulation of gene expression (McEwen 1995).

Now, cooccupancy may in some cases stimulate and in other cases inhibit gene expression. Other interactions between regulatory proteins include the tethering of one DNA-binding protein to another. In addition, the affinity between a glucocorticoid receptor and a fos-jun heterodimer is so high that both proteins are removed from interacting significantly with their respective response elements. So, what started as a relatively simple picture of a circulating hormone entering a cell and binding to specific receptors has ended with the notion that neurotransmitters and second-messenger systems also regulate genes and sometimes do so in combination with circulating hormones (McEwen 1995).

The role of steroid hormones is even more complicated. In addition to the intracellular receptors, we now recognize at least two kinds of membrane receptors, mainly through actions and not so much through binding (McEwen 1995; Paul and Purdy 1992; McEwen 1991; Gee 1988). One of them is the binding of derivatives of progesterone and desoxycorticosterone to the GABA receptor, which acts very much like a barbiturate or benzodiazepine but through a different site to enhance the passage of chloride ions through the channel (figure 2).

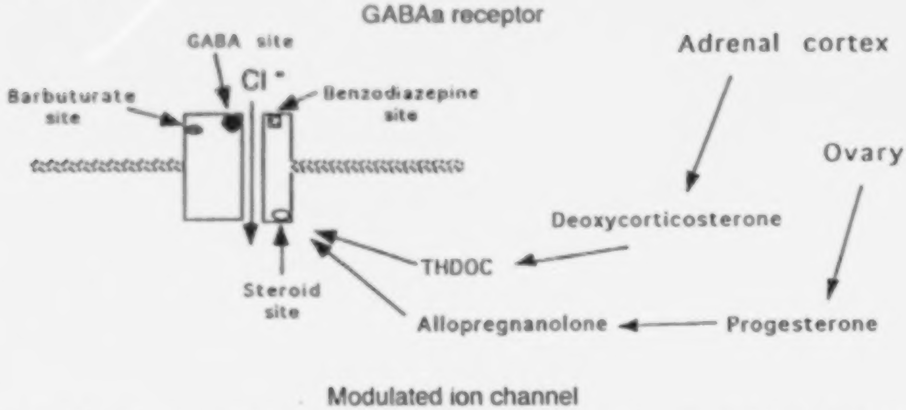

Figure 2 Steroid metabolites of progesterone and deoxycorticosterone bind to sites on the GABAa receptor and regulate chloride ion flux along with GABA, benzodiazepines, and barbiturates. This pathway is one of the so-called "nongenomic" effects of steroids on target cells, and it accounts for the anaesthetic effects of high doses of progesterone and lesser amounts of related steroids, like the anaesthetic steroid, alphaxolone.

Another is an example described in the newt, *Taricha granulosa*, by Moore and Orchinik (Orchinik et al. 1994) that involves a glucocorticoid receptor on membranes that is coupled to a G protein and regulates rapidly the stress-induced inhibition of reproductive behavior in that species. Such receptors have not been definitively identified in other species, but again, binding assays that were successful in *Tarichs* are very difficult to apply to all of the membrane receptors. Thus, we mostly have functional evidence for rapid membrane effect of steroids, and the idea that second messengers are involved in some cases provides yet another pathway for steroid action to affect genomic events.

Developmental Processes and the Role of Hormones

This section covers three topics having to do with developmental actions of hormones (figure 3). One of them is sexual differentiation of the brain, where

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A. Sexual differentiation

Male:	Perinatal testosterone secretion	Brain circuits masculinized	Male brain and behavior
Female:	minimal gonadal activity	Female circuits develop without interference	Female brain and behavior. Ovarian cyclicity.

B. Thyroid hormone

Thyroid hormone	Hyperthyroid Euthyroid Hypothyroid

C. Stress

Prenatal glucocorticoid secretion	Increased HPA reactivity	Increased brain (esp. hippocampal) aging
Postnatal thyroid hormone secretion	Decreased HPA reactivity	Decreased brain (esp. hippocampal) aging

Psychosocial stress—a potent stressor

- Cognitive impairment
- Behavioral reactivity—substance abuse
- Health consequences—more disease at lower rank?

Figure 3 Schematic summary of three principal hormone-directed pathways affecting brain development.

the presence of testosterone in mammals during early phases of development, usually in utero but perinatally in the rat, is responsible for the masculinization and defeminization of the male brain and behavior from what is otherwise a basically feminine pattern. The second system has to do with thyroid hormone. Here, the basic plan of development appears to be the happy medium, that is, the euthyroid state. Either hyperthyroid or hypothyroid conditions can cause neural development to deviate. The question is the degree to which and the timing in which these effects of thyroid hormone are reversible or irreversible.

The third topic has to do with the development of stress responsiveness. The basic notion is that events can happen early in development, often prenatally, sometimes postnatally, which can increase the reactivity of the stress hormone axis, the hypothalamic-pituitary-adrenal (HPA) axis, and also, to some extent, the sympathetic nervous response to external events that we often call stressors. Moreover, if stressful events take place prenatally, they permanently increase the activity and responsivity of the system; conversely, early postnatal experience that buffers stress has the effect of decreasing for life the reactivity of the stress hormone axis.

Sexual Differentiation

In sexual differentiation, the main role of genes is to determine the sex of the gonads. Thereafter, the quality and quantity of the sex hormones produced by the testes and ovaries at different stages of life interact with experience to produce phenotypic sex differences (Goy and McEwen 1980). Beginning with studies on experimental animals, it has been recognized that the brain undergoes subtle changes as a result of sexual differentiation. There are sex differences in the size and cellularity of hypothalamic nuclei as well as nuclei in the spinal cord innervating the penis. Both the anterior commissure and the corpus callosum itself show gender differences in shape and size as well as differences related to handedness or laterality. Animal studies indicate that many of these differences can be programmed or reversed by either giving testosterone to embryonic females or inhibiting testosterone secretion in embryonic or newborn males (McEwen 1995). In humans, functional imaging of the brain has revealed sex differences that relate to laterality of function (Gur et al. 1995).

Two examples illustrate the consequences of sexual differentiation. The ventromedial hypothalamus of the female rat has cyclic formation and breakdown of synapses on neurons in the ventral medial nucleus (VMN) that are estrogen sensitive and have estrogen-inducible progesterone receptors. The synaptogenesis is regulated during the estrus cycle so that there is increased production of synapses by the time of proestrus when ovulation and sexual behavior occurs. These synapses disappear rapidly between the day of proestrus and the day of estrus when the cycle begins again (Frankfurt et al. 1990).

The male is different; even if you castrate a rat and give it a dose of estrogen that will induce synapses in the female, no synapse induction occurs on the VMN neurons (Segarra and McEwen 1991). Moreover, the male tends to have more synapses on these neurons to begin with (Matsumoto and Arai 1986). It is not clear where these synapses come from or how the afferent projections differ from those of the female; the basic difference in the estrogen sensitivity is the fundamental point. The male VMN neurons have estrogen receptors just as in the female VMN, but estrogen treatment induces fewer progestin receptors in the male than in the female VMN (Brown et al. 1987; Rainbow et al. 1982). So, this sex difference appears to be more than simply the absence of gonadal hormone sensitivity and involves, instead, some aspect of developmental programming of how the cell responds to these exogenous hormones.

At the same time that we found the estrogen regulation of synapses in the VMN, a graduate student in our laboratory, Catherine Woolley, discovered that the CA1 region of the hippocampus shows a similar pattern of cyclic synaptogenesis on dendrites (Woolley et al. 1990). She then used electron microscopy to reveal that the new spines are occupied by synapses (Woolley and McEwen 1992). Both spines and synapses rapidly downregulate between the day of proestrus and estrus, and this event repeats itself every 4 or 5 days during the estrous cycle of a female rat.

Two aspects of this synaptogenesis are especially interesting. First, although synapse formation is induced by estrogen, and synapses are caused to disappear by giving progesterone, NMDA receptors play a key role. NMDA receptor blockers given with estrogen prevents new synapse formation (Woolley and McEwen 1994). It also appears that the CA1 pyramidal neurons do not have intracellular estrogen receptors, and the only estrogen receptors in the adult hippocampus are found in interneurons that have projections onto large numbers of these pyramidal neurons (DonCarlos et al. 1991; Loy et al. 1988). Moreover, there are estrogen-sensitive projections to CA1 from the serotonergic system and the entorhinal cortex, but we do not know if they play a role in synaptogenesis. So, there may be a transsynaptic process in which either a genomic estrogen receptor is involved in some other cell type or some kind of nongenomic mechanism on these cells collaborates with the excitatory amino acids.

The type of sexual differentiation seen in the hypothalamus also occurs in the hippocampus; for example, male rats do not show estrogen-induced synaptogenesis on hippocampal pyramidal neurons. If you treat female rats with estrogen at birth, or with testosterone, this defeminizes the female and makes it male-like in its inability to respond to estrogen treatment. Conversely, if you block the aromatization of testosterone at birth in newborn males, it makes the hippocampus responsive in adult life to estrogen administration for synapse induction (Lewis et al. 1995). A transient expression of elevated estrogen receptor levels in the hippocampus as well as aromatizing enzymes probably mediate this developmental event (MacLusky et al. 1987; O'Keefe et al. 1995; O'Keefe and Handa 1990).

Functionally speaking, this means that male rats have an increased capacity to learn a spatial task in a Morris water maze or a radial arm maze when global cues are present. Males learn the task faster than females, and neonatally estrogen-treated female rats also show a more rapid acquisition (Williams and Meck 1991). Thus, spatial learning is affected, and we know that spatial learning is a function of the hippocampus.

This emphasizes the important point that more than reproductive events are influenced by sex hormones and by sexual differentiation. The noradrenergic, serotonergic, basal forebrain cholinergic, and various dopaminergic systems all show evidence of ovarian hormone sensitivity and may also show evidence of some degree of sexual differentiation (McEwen et al. 1995*b*). Thus, sex differences are more widespread than we had heretofore believed.

What does this mean in human terms? There is a reported sex difference as a function of age in Alzheimer's disease (Birge 1994). From ages 55 to the late 80s, there is an increasing incidence of Alzheimer's disease in women compared to men, although both sexes show an increasing frequency. We also know that there are sex differences in developmental learning disorders, with males having 3–4 times higher frequency (Hier 1979). Moreover, in major depression, women outnumber males by somewhere between 2 and 3 to 1, but men, in turn, outnumber women in the frequency of substance abuse and antisocial behavior (Regier et al. 1988). So, there are gender differences that may be traceable in part to the hormonal and structural plasticity that I have described.

Thyroid Hormone

The second endocrine system involved in brain development has to do with thyroid hormone. While early work looked at hypothyroidism, a lot of recent work has dealt with hyperthyroidism. A number of years ago, Gould (Gould et al. 1991) carried out an experiment dealing with transient exposure of newborn male and female rats to thyroid hormone during the time when testosterone is secreted in newborn males. These treated and control rats were then examined after sexual maturity to look at various parameters of structure and neurochemistry in the hippocampus and basal forebrain.

One of the effects was to increase the length and size of dendritic branches of CA3 pyramidal neurons in the hippocampus (Gould et al. 1990*b*). The thyroid hormone treatment also increased the density of spines on dendrites of CA3 pyramidal neurons, as determined by the Golgi procedure. CA1 pyramidal neurons were not affected. Astroglia in the basal forebrain were also hypertrophied, and there were increases in expression of cholinergic markers in basal forebrain, particularly in males (Gould et al. 1990*a*).

Another sexually differentiated phenomenon was the effect of thyroid treatment in the neonate on parameters in the medial septum (Westlind-Danielsson et al. 1991). Neuron cell body area became smaller in the treated males, but became somewhat larger in the treated females. However, in both the basal forebrain and septal cholinergic systems and the CA3 pyramidal neurons, there was an intrinsic sex difference that did not go away with thyroid hormone treatment, but rather was enhanced, in that males appeared to respond more strongly than females to the neonatal thyroid hormone treatment. Other investigators have reported that the dentate gyrus of male rats appears to be larger than the dentate gyrus of female rats (Roof 1993).

Now, what does this mean functionally? Bigger is not necessarily better, and much to our surprise, looking at learning in a radial arm maze, thyroid hormone-treated rats made more errors and took a longer time to complete the task (Pavlides et al. 1991). On each measure, the treated animals did worse, although ultimately they achieved the same criteria as the controls.

There is, however, a study on mice that appear to be intrinsically hypothyroid at birth and show a poorer learning ability than other closely related strains (Schwegler et al. 1991). Giving thyroid hormone to these mice at birth seemed to correct the deficit and improve learning ability in adult life. Thus, it comes back to this happy medium idea of thyroid hormone action—the brain needs a certain amount for normal neural development and either too much or too little is bad.

Stress and Stress Hormones

In our laboratory, study of the stress hormone axis is focused on the hippocampus, which we showed a number of years ago has receptors for circulating adrenal steroids (McEwen et al. 1968). Both the pyramidal neurons of Ammon's horn and the granule neurons of the dentate gyrus have these receptors (Herman et al. 1989; Gerlach and McEwen 1972). Now, we are studying aging of the brain, where the presence and function of adrenal steroid receptors in hippocampus has become an important issue (Landfield and Eldridge 1994; Sapolsky 1992; Sapolsky et al. 1986).

Aging is a developmental process in which early experiences have life-long effects. Studies from the Meaney laboratory (Meaney et al. 1988) have shown that some individual aged animals are severely impaired in their ability to learn a task in a Morris water maze. Unimpaired aged animals perform just as well as young animals. In trying to understand the phenomenon of individual differences in aging, Meaney and colleagues looked at corticosterone secretion during a stressful experience and found that the age-impaired animals continued to produce high levels of glucocorticoids during and after a period of stress, whereas the young and age unimpaired animals shut off the corticosteroid secretion quite efficiently after the stress was over. Now, we need our adrenal cortex to survive stressful experience, but once we have had the hormonal stress response, it is very much in our interests to shut if off, because persistent secretion can cause problems.

Many years ago, Denenberg, Levine, and Ader (Ader 1968; Denenberg and Haltmeyer 1967; Levine et al. 1967) devised a procedure, called "handling," that consists of briefly removing rat pups from the mother for 10 minutes per day during the first 2 postnatal weeks. This early handling reduced the reactivity of the HPA axis for life.

This effect was demonstrated recently in a collaboration between Meaney and Plotsky (Meaney et al. 1994). Meaney and Sapolsky showed a number of years ago that handling rats at birth reduced the rate of brain aging and resulted in fewer of the age-impaired rats found in normal rat populations (Meaney et al. 1988). Their conclusion was that handling, by reducing HPA activity, reduces life-long exposure to adrenal hormones and slows the course of hippocampal aging.

Recently, studies from the group of Simon and LeMoal in Bordeaux (Dellu et al. 1994) revealed that animals with a hyperactive HPA axis in response to novelty show a more rapid rate of brain aging, as measured by spatial learning. The cause of that hyperactivity is not indicated.

Regarding hormonal involvement, a number of laboratories have proposed that glucocorticoid exposure may be the mediator of the enhanced HPA reactivity during early development. There are also indications that thyroid hormone may have some role in reducing the reactivity of the HPA axis to handling. Whether that is the case or not, it does appear that experimenters can produce life-long individual differences in animals, either increased or decreased reactivity of the stress hormone axis. This has a number of life-long consequences, one of which seems to be that animals with increased reactivity show increased aging of the brain, especially the hippocampus.

Although I have emphasized the adrenal steroids in changes going on in the hippocampus, it would be a mistake to assume that they act alone. Indeed, the excitatory amino acids are also prominently involved (McEwen et al. 1995*a*). Lowy and collaborators (1993) a number of years ago showed that, during restraint stress using in vivo microdialysis, there is glutamate release in the hippocampus and no release of taurine, a control amino acid that is not synaptically localized. They also found that adrenalectomy of these animals reduced the amount of glutamate released during restraint stress, suggesting that some adrenal factor, very likely adrenal steroids, are responsible for facilitating the ability of excitatory synapses in the hippocampus to release glutamate during stressful challenge.

Recently, Lowy found that, in aging animals, glutamate is released during stress, but when the stress is over, the glutamate does not shut off (Lowy et al. 1995). It tends to remain quite high for a period of time, suggesting another kind of dysregulation similar to the prolongation of glucocorticoid secretion after stress seen in aging rats and prenatally stressed rats. Landfield and collaborators found that aging rats show an enhancement of voltage-activated calcium channel activity (Landfield and Eldridge 1994). Thus, there appears to be a dysregulation of glucocorticoid secretion, glutamate release, and calcium channel activity that may be interconnected.

What are the most direct manifestations of repeated stress in the hippocampus? Our own recent work has indicated that repeated psychosocial stress, such as being a subordinate in a social hierarchy; repeated restraint stress; and repeated glucocorticoid treatment in young adult rats will each cause apical dendrites of CA3 pyramidal neurons that receive the mossy fiber innervation from the dentate gyrus to undergo a reversible atrophy, loss of branching, and loss of total dendritic length (McEwen et al. 1995*a*). The basal dendrites do not change. Although this atrophy can be driven by glucocorticoid treatment, each of these causes of dendritic atrophy are blocked by Dilantin, which is an antiepileptic drug that blocks sodium channels and T-type calcium channels and interferes with glutamate release and some of the postsynaptic actions of glutamate. Dendritic atrophy is also blocked by blocking NMDA receptors. Thus, it appears that the final common path involves excitatory amino acids and NMDA receptors and that the glucocorticoids are involved in facilitating certain aspects of the neural activity within the hippocampus.

In the human brain, the application of MRI to a variety of clinical disorders with some cognitive impairment and a connection either to stress, glucocorticoids, or aging has revealed atrophy of the hippocampus on the order of 10–15 percent (McEwen and Magarinos 1996; Sapolsky 1996). Although most extreme in Alzheimer's disease, hippocampal atrophy has been seen in Cushing's syndrome with elevated glucocorticoids and appears to be a reversible, correctable disorder (Monica Starkman, personal communication). It has been seen in recurrent depression and in posttraumatic stress disorder (McEwen and Magarinos 1996; Sapolsky 1996).

Moreover, atrophy of the human hippocampus has been seen in normal aging. A recent study from Lupien, along with Meaney and Deleon (Lupien et al. 1996), has revealed that aging individuals who show a progressive increase in their baseline cortisol assay levels over 4 successive years and some cognitive impairment related to the temporal lobe actually have on examination a 12–15 percent smaller hippocampus than comparably aged individuals who do not show cortisol elevation and cognitive decline.

Stress and Adaptation—The Concept of Allostatic Load

In all of these cases, we need to know how reversible or treatable are these changes in hippocampal volume? Do they represent permanent cell loss or some kind of atrophy that can be treated? Does this type of atrophy either protect the hippocampus or lead to later permanent damage? We do not know the answers yet, but these questions bring me to a concept of adaptation to stress and the cost that adaptation can have to the body.

Systems of the body like the HPA axis and the output of neurotransmitters like excitatory amino acids become activated under challenge, and their actions enable cells and organs to adapt. With external demand, the activity of these systems increases, and although we need them to survive, we have to turn them off again when they are not needed.

The actions of these systems to promote adaptation are called "allostasis" or "achieving stability through change," that is, allostasis helps to maintain homeostasis (Sterling and Eyer 1988). When allostatic systems are not turned off, the excess activity produces wear and tear that we call "allostatic load" (McEwen and Stellar 1993; McEwen 1998). When allostatic load occurs for a long time, say for a lifetime in prenatally stressed rats with a hyperresponsive HPA axis, then the wear and tear manifests itself as earlier cognitive decline and more rapid hippocampal aging.

Rats that have been handled at birth and show less reactive HPA axes experience less allostatic load and slower brain aging. Thus, the developmentally programmed reactivity of an allostatic system determines the degree to which that system will show allostatic load.

Another determinant of allostatic load is the number of stressful encounters, and this will produce wear and tear whether or not the allostatic system is hyperresponsive. We know, for example, that in animal models, a situation involving unstable dominance hierarchies increases activity in the individuals vying to maintain their dominant position. Social isolation and maternal deprivation increase activity of allostatic systems, as do hostility and other types of conflict, whereas affiliative behaviors and social support in general decrease activity. So, we have to consider the intrinsic reactivity of the allostatic system itself, how well it can shut off, and the social environment that ultimately controls whether and how often the allostatic system is turned on.

Type-2 diabetes is one example of allostatic load. This type of diabetes is associated with insulin resistance and increased levels of glucocorticoids and catecholamines and so forth. Genetic factors and nutrition and diet, along with stressful experiences, interact with each other (McEwen and Stellar 1993). Together, these forces can lead to a vicious cycle and promote pathology involving hypertension, diabetes, obesity, and atherosclerosis.

Regarding atherosclerosis, the Bowman Gray group has studied male cynomolgus monkeys in unstable dominance hierarchies where they have to repeatedly vie for position. These animals show accelerated rates of atherosclerosis (Manuck et al. 1988). Their diet is also a determinant of the cardiovascular disease progression.

Another example of stress and disease, this one having to do with type-1 diabetes, that is, autoimmune insulin-dependent diabetes, is an experiment on the BB rat, which is genetically susceptible to this disorder. When these rats were stressed daily for 14 weeks starting at about 4 weeks of age, they showed an increased incidence of the disease—in males, 80 percent by week 10 of stress compared to 50 percent without stress, and in females, 70 percent by week 12 versus 50 percent in unstressed controls (Lehman et al. 1991). Thus, for this example of a genetic trait, the repeated exposure to a series of stressors rotated every day over 14 weeks increased the incidence of the disorder.

How does the immune system respond to stress? In a recent Ph. D. dissertation at Rockefeller, Dhabhar found a stress effect that may help explain what happens in the BB rat. He used the delayed-type hypersensitivity (DTH) response, in which a rat is exposed to a simple antigen to develop an immunologic memory. Later, he challenged one ear of the animal and measured ear thickness as a function of time after this challenge. The ear thickened from an invasion of immune cells. When he applied stress at the time of the challenge, he found that the ear became even thicker, indicating that even more cells were invading (Dhabhar and McEwen 1996). This enhanced thickening can be mimicked by giving glucocorticoids in a moderate dose, and it can also be blocked by blocking glucocorticoid synthesis. In the BB rat experiment, the immunologic memory exists in the form of immune cells that attack the pancreatic islet cells, which produce insulin. According to Dhabhar's DTH model, daily stress may cause more of these cells to enter the pancreas and destroy the insulin-producing capacity.

Delayed-type hypersensitivity is a model for many kinds of immune responses where there is an immunologic memory. In allergies or autoimmune disorders, such a memory is bad, and stress can make things worse. However, in fighting cancer and infectious disease, a DTH response is an important defense mechanism. A stress-enhanced DTH response is somewhat counterintuitive, because conventional wisdom says that stress causes immunosuppression. However, that is only part of the story, and, in fact, the allostasis associated with the stress response is actually beneficial when the immune system is reacting to a dangerous pathogen.

Another important point is that exogenous steroids, such as dexamethasone and high doses of corticosterone, will inhibit the DTH response (Dhabhar, personal communication). Dexamethasone bypasses the corticosteroid binding globulin (CBG) that normally buffers endogenous adrenal steroids from gaining access to immune cell glucocorticoid receptors, whereas high doses of corticosterone simply overwhelm the CBG capacity.

Conclusions

We need to recognize the importance not only of the intrinsic reactivity of a system that may be genetically or developmentally determined, but also of the frequency of stressful events in relation to social support and social conflict. Furthermore, we must not forget about important traits like optimism, pessimism, and a sense of control that influence physiological reactivity of allostatic systems.

These traits bring us back to the brain and the limbic system. In trying to understand how early experiences can affect emotionality, we have to consider the amygdala, which is a brain structure involved in fear conditioning and the memory of unpleasant events, and the hippocampus, which provides memory of recent events and the context in which unpleasant or emotionally charged experiences occur. Takahashi at the University of Wisconsin and Gould at Rockefeller are studying the dentate gyrus, which forms postnatally during the 2 weeks when handling is effective in producing the effects described earlier. As the dentate gyrus forms, local neurogenesis occurs that is under control of both adrenal steroids and excitatory amino acids acting through NMDA receptors. Takahashi (1995) has studied the development of behavioral inhibition, or fear of strangers, that appears when the rat pups' eyes are opened and lasts until about 30 days of age. He has found that behavioral inhibition does not occur as strongly in adrenalectomized animals. However, when he implants tiny crystals of corticosterone in the dentate gyrus, he can restore normal levels of behavioral inhibition.

Gould has found that blocking NMDA receptors has the same effect as adrenalectomy (ADX), namely, to prevent the development of behavioral inhibition. Conversely, giving NMDA as an agonist, or giving excess glucocorticoids, enhances the development of behavioral inhibition (Cameron and Gould 1996). These effects parallel what happens to neurogenesis—ADX or NMDA blockade increases granule cell turnover, whereas NMDA and excess glucocorticoids suppress granule cell birth and death and stabilize the neuronal population. It is thus conceivable that behavioral inhibition is a manifestation of the maturation of granule neurons and their formation of stable connections with other nerve cells within the hippocampus.

Similar things may be happening in the adult dentate gyrus, where ongoing neurogenesis is enhanced by both adrenalectomy and blocking NMDA receptors (Cameron et al. 1995). Entorhinal cortex lesions remove excitatory input to the dentate gyrus via the perforant pathway, and such lesions also increase dentate gyrus neurogenesis. Interestingly, unlike ADX, blocking NMDA receptors enhances cell birth but does not seem to increase cell death. Adrenal steroids may be modulating some aspects of the NMDA receptor expression, and that may be the connection between them. However, removal of adrenal steroids evidently turns on a cell death gene, and this gene is not repressed by NMDA receptor activation.

In the adult dentate gyrus of both the rat and the tree shrew, stressful experiences rapidly inhibit neurogenesis (Gould, Galea, and McEwen, unpublished). This is true for a rat experiencing the odor of a fox, a natural predator. It is also true of the tree shrew experiencing conflict with a dominant conspecific in a resident-intruder paradigm. We can speculate that inhibition of neurogenesis stabilizes a population of neurons that results in retention of the context of a fear-related event.

How does this relate to early experiences and the effects of prenatal stress and postnatal handling, which seem to have such a powerful role in determining life-long patterns of emotionality? It will be important to find out if such events affect neurogenesis in the dentate gyrus and how this, in turn, alters the ability of the dentate gyrus to respond to stressful events later in life. For example, early experiences may affect the interconnections between the hippocampus and the amygdala.

Megan Gunnar points out that, in human infants, the period right after birth is a time of great lability of the HPA axis. In fact, the establishment of a stable relationship with the mother is extraordinarily important in making the HPA axis refractory to many kinds of stressful challenges. There may be a parallel with the stress hyporesponsive period in the newborn rat, when maternal buffering protects the infant from many types of stressors.

The final point is to reemphasize that what happens early in life, be it related to sexual differentiation, the actions of thyroid hormone, or stressful early experiences, has a life-long impact and may affect the appearance of many forms of psychopathology and physical pathology as well as the rate of brain aging.

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Biobehavioral Development In Prenatal Life: Basic Principles

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ABSTRACT

This chapter introduces principles of prenatal neurobehavioral development. The purpose is to provide a conceptual context for future investigations of the human fetus. Five basic principles cover the most salient features of neuromotor development in late gestation. These principles are thought to provide an integrative perspective for postnatal development in the behavioral and cognitive spheres.

The search for noninvasive methods to study perinatal life continues. This search is being accompanied by a parallel focus on systematic conceptual models of fetal development. Prenatal research has inherent technical and theoretical challenges that require conceptual synthesis. The study of prenatal neurobehavioral development in the human reveals a relative paucity of empirical studies and conceptual models. Progress in these areas is necessary in helping to guide research and provide direction for future investigations. Thus, the goal of this brief chapter is to articulate some basic principles of neurobehavioral maturation that are characteristic of prenatal life. These principles will help augment existing efforts to understand the role that prenatal experience plays in postnatal functioning.

Much of prenatal research is driven by questions specific to the hypotheses of a particular study. Thus, several lines of inquiry have developed. These include, most recently, studies of prenatal sensory development in the rat fetus (Smotherman and Robinson 1988), prenatal behavioral development and human voice discrimination and perception (DeCasper and Fifer 1980; Fifer and Moon 1989), habituation (Kisilevsky et al. 1992; DiPietro 1996*a, b*), and fetal responses to labor (Emory et al. 1982*a, b*; Emory and Noonan 1984*a, b*). There are now conceptual discussions in the obstetrics literature about the importance of fetal behavior in clinical diagnosis (Hepper and Shahidullan 1992; Hepper 1996) and fetal growth and blood flow velocity in relation to

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neurological morbidity and intelligence at age 7 (Rizzo et al. 1996; Ley et al. 1996). Most recently, Emory and Israelian (in press) have offered a cognitive perspective on prenatal development.

The evolution of prenatal research, especially in the human, has incorporated contemporary technologies that allow closer scrutiny of functional development and provide a new perspective on fetal behavior. This evolution is incomplete until formal principles of prenatal neurobehavioral development are articulated and confirmed by empirical investigation. As a starting point for perspective building, this discussion briefly outlines five principles that appear to govern much of human prenatal behavioral development during the latter stage of pregnancy (e.g., late second and third trimesters).

A Shift From Endogenous to Exogenous Drives

An overriding principle that seems to differentiate prenatal from postnatal life is the characteristic developmental shift from endogenous to exogenous drives. The human fetus gradually undergoes a transition from intrinsically internal to externally driven influences. These influences direct the course of prenatal neurobehavioral development and, subsequently, postnatal behavior. This shift from endogenous to exogenous influences means inborn or genetic programs assume a diminishing role in phenotypic expression. Stimulus response and experiential factors assume an increasing role. Prenatal maturation is indexed by this shift, but it does not demarcate a complete change from one set of influences to another. One of the best examples of this principle is the onset of heart rate and the emergence of spontaneous behavior during embryonic life. Both phenomena become increasingly regulated by external stimulation.

Anticipatory Action and Motor Primacy

The second principle is one of behavioral organization, which includes principally anticipatory action and motor primacy. The manifestation of this principle is reflected in the fairly pervasive sort of anticipatory or preparatory aspects of embryonic behavior that are crucial for later adaptive functioning. A key element of this principle is that some of these adaptive functions appear well in advance of their necessity for survival (Preyer 1885, 1890). These include reflex phenomena such as grasping, sucking, and breathing movements in utero.

Higher level processes are also manifested in fetal life, such as habituation responses and preference for the maternal voice (Leader and Baillie 1988; Fifer and Moon 1989). These behaviors and responses can be viewed as properties of the fetal nervous system that prepare it for postnatal life. In addition, anticipatory and motor primacy features reflect both facilitative precursors—those that exercise a direct and specific quantitative or temporal influence on later development—and deterministic precursors that tend to force or channel neurobehavior in one direction or another. These ideas were eloquently expressed by Gottlieb (1976).

Neuromotor Integration and Motor-Action-Template

A third principle that characterizes human prenatal life is neuromotor integration and the formation of a motor action template (Emory and Israelian in press). Neuromotor integration is conceptualized as a drive state reflecting a need of the fetus to economize physiological expenditures and prepare for the challenges of an extrauterine environment. It is represented by (1) behavior that tends to include a predominance of isolated movements early in gestation, gradually giving way to coordinated movement of several muscle groups; (2) a tendency for nonlinear progression in behavioral and other developmental functions; and (3) emergence of coordinated patterns that seem to have been suppressed early and later emerge. Figure 1 illustrates isolated movements which are features of fetal behavior that are more likely prior to completion of neuromotor integration in the younger fetus and which are negatively correlated with advancing fetal age. Figure 2, in contrast, is composed of behavior epochs and episodes, which have a significantly longer duration than isolated fetal movements and are positively correlated with maturational indices.

An especially intriguing feature of fetal behavior and maturation is the associated correlations between cerebral blood flow during gestation and the expression of different features of fetal behavior. As one can see from figure 3 (see page 17), cerebral blood flow in the major arteries of the Circle of Willis has a linear relationship with gestation from about 25 weeks to 38 weeks. Fetal cerebral blood flow velocity is more negatively correlated with isolated or random fetal movement and more positively correlated with organized fetal behavior.

Biobehavioral Synchrony

The fourth principle is one of biobehavioral synchrony. This principle is reflected in the coupling of previously isolated physiological and behavioral phenomena. An excellent example of this principle is the emergence of heart rate and behavior coupling following neuromotor integration. Prior to the stage of neuromotor integration (e.g.,<28 weeks), there is unsynchronized behavior or lack of coupling between movement and heart rate responses. This uncoordinated feature of physiological and behavioral responding in the fetus indexes fetal maturity.

Biobehavioral synchrony as a principle underlying fetal development allows investigators to index important developmental milestones, and also facilitates

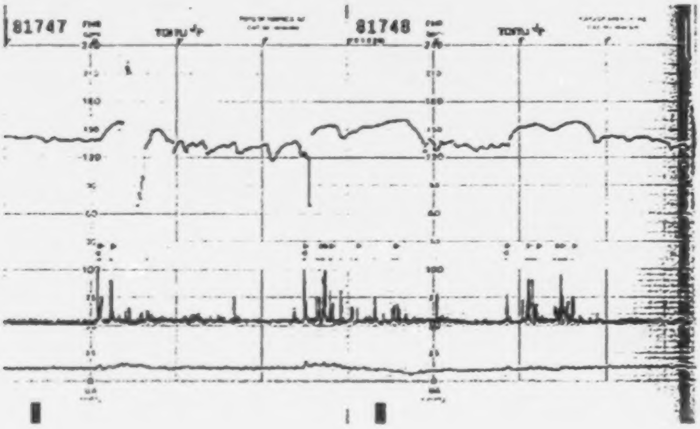

Figure 1

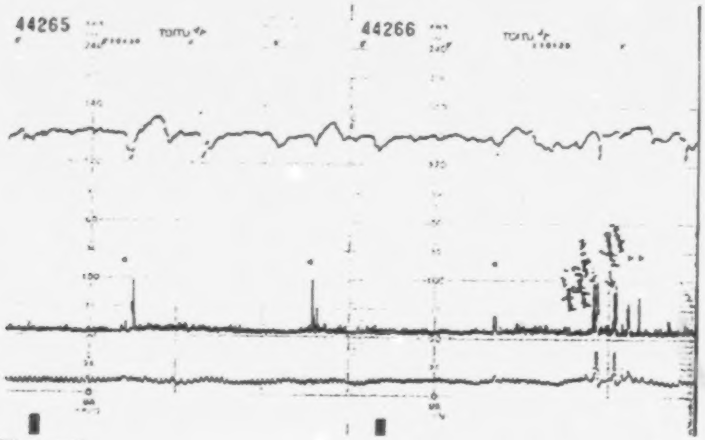

Figure 2

clinical assessment of fetal integrity and capacity for tolerating the stress of birth. During antenatal assessment (prenatal assessment), the nonstress test is typically used as an early screen for possible neurological problems or fetal vulnerability. Historically, the coupling of fetal movement with fetal heart rate change has been seen as indicative of a reactive and reassuring sign of fetal maturity. The lack of fetal heart rate change during the nonstress test and the need for close monitoring may signal a vulnerable fetus.

The principle of biobehavioral synchrony is implicitly used in clinical settings to help the clinician make judgments and decisions about intervention. Biobehavioral synchrony as a principle allows the behavioral perinatologist to focus upon integrated aspects of fetal responsivity that index nervous system maturation. This principle represents one of the hallmarks of prenatal neurobehavioral development and underlies the capacity for organized behavior.

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Inhibitory Control and State Regulation

The last principle to be discussed is inhibitory control and state regulation. This principle represents ongoing maturational processes that occur prenatally. A cornerstone of organized behavior and maturation in the fetus is the emergence of identifiable states of arousal and the capacity to inhibit or suppress behavior.

This final stage of fetal development is clearly defined by rest-activity cycles that are increasingly observable. They are accompanied by suppression of sympathetic drives and the changing nature of organized behavioral actions. For example, baseline fetal heart rate tends to show a progressive decline as term approaches. Moreover, the changing architecture and distribution of arousal states correlates with decreasing baseline heart rate during gestation.

Inhibitory control is based in part on the increasing parasympathetic influence that suppresses heart rate and other sympathetically driven activity. Fetal heart rate is controlled principally by rate increases secondary to the need for oxygenated blood. There is virtually no variation in stroke volume during early fetal life, and thus metabolic needs are met by increasing heart rate. In the more mature fetus and infant, vagal influences contribute to an ability to modulate stroke volume, and therefore, oxygen-driven fetal heart rate increases.

Conclusion

The maturing fetus will go through a number of different developmental stages in which behaviors have previously been uncoordinated, isolated, and uninhibited. The attempt here is to integrate these aspects of fetal life into organized principles that underlie human development. One focus has been to characterize the unborn fetus as a responsively viable organism and to lay a conceptual foundation for neonatal patterns that are typically seen in the infant.

These biobehavioral principles are derived from observation and experimentation with the intact or uncompromised organism. Although the principles may apply to all mammalian species, the primary focus here is on the human. The effects of ominous or clinically risky situations on the development of behavioral patterns is a topic for another discussion. However, conditions that compromise the intrauterine environment may realign the orderly progression of developmental stages, and thus modify functions that characterize the principles discussed here. Indeed, in clinical obstetrics with high-risk populations, some fetuses may show advances or delays in the emergence of certain neurobehavioral functions.

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Early Experience, Emotion, and Brain: Illustrations from the Developmental Psychopathology of Child Maltreatment

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Investigations conducted within a developmental psychopathology framework, with its focus on the mutually enriching interchanges that occur between the study of normal and abnormal development and with its attention to biological as well as psychological influences on the course of ontogenesis, offer a powerful context within which to conceptualize the relations among neurobiological development, experience-dependent processes, and psychopathology. Common themes emerge as psychological and biological perspectives on normal and deviant human development are examined. In particular, the dialectic that exists between the canalization of ontogenetic pathways and the belief that continued opportunity for change exists throughout the life course are acknowledged by scientists conducting research in the area of developmental plasticity (Cicchetti and Tucker 1994*a*,*b*).

Rather than adhering to a unidimensional belief in the deterministic role that unfolding biology exerts on behavior, a number of investigators operating within the developmental psychopathology tradition have asserted that brain development and function and their subsequent influence on behavior possess self-organizing properties that can, in fact, be altered by experiences at certain sensitive periods of development that occur across the life course (Cicchetti and Tucker 1994*a*; Eisenberg 1995). Moreover, this framework moves beyond controversies associated with nature versus nurture to a more integrative and dynamic transactional view on development that stresses the importance of both psychological and neural self-organization.

Child Maltreatment

In this chapter, research conducted with maltreated infants and children is

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used as an exemplar of the impact of traumatic experiences upon psychological and biological developmental processes. Because maltreated children experience the extremes of caregiving casualty, they provide an opportunity for scientists to discover the myriad ways in which environmental and psychological stressors can effect biological and behavioral systems. Comparisons between maltreated and nonmaltreated children can elucidate our understanding of the caregiving processes that contribute to the development of regulated neurobiological and behavioral systems.

The Organizational Perspective on Development

Our research on the sequelae of child maltreatment has been guided by the organizational perspective on development (Cicchetti and Sroufe 1978; Sroufe 1979). Organizational theorists believe that, across the developmental course, the evolving capacities of individuals and their active choices allow for new aspects of experience, both internal and external, to be coordinated in increasingly complex ways.

At each developmental transition, individuals are confronted with specific developmental tasks that are central to that period. From infancy through adulthood, new salient developmental issues (i.e., sensitive periods) emerge and are of primary importance during their particular stage of ascendance. However, despite subsequent developmental issues gaining greater salience, each developmental task remains an issue of lifespan significance (Cicchetti and Schneider-Rosen 1986). Through differentiation and hierarchic integration, the resolution of each stage-salient issue is coordinated with the prior organization of developmental systems, and reorganization occurs, moving the individual's development forward. The quality of the resolution of each stage-salient issue primes the way subsequent developmental issues are likely to be negotiated. Through their active role in the ontogenetic process, individuals proceed down different developmental pathways.

For example, inadequate resolution of developmental challenges may result in a developmental lag or delay in one of the biological or behavioral systems. As a result, less than adequate integration within that domain will occur, and that will compromise adaptive integration across domains as hierarchical integration proceeds. Thus, incompetence in development may be viewed as a problematic integration of pathological structures. Over time, difficulty in the organization of one biological or behavioral system may tend to promote difficulty with the way in which other systems are organized as hierarchical integration between separate systems occurs. The organization of the individual may then appear to consist of a synthesis of poorly integrated component systems.

Although early incompetent functioning tends to promote later incompetence, because the individual arrives at successive developmental stages or transitions with less than optimal resources available for responding to the challenges of that period, this progression is not inevitable, but probabilistic.

Changes in the internal and external environment may lead to improvements in the ability of the individual to grapple with developmental challenges, resulting in a redirection in the developmental course. Thus, while historical factors canalize and constrain the adaptive process to some degree, plasticity is possible as a result of adaptive self-organization.

Child maltreatment affects the development of several illustrative stage-salient developmental issues: affect regulation, the development of a secure attachment relationship, and the ontogenesis of autonomous self-system processes. (For a fuller elaboration of how child maltreatment affects these and other developmental processes, see Cicchetti and Lynch 1995 and Cicchetti and Toth 1995).

Affect Regulation

Conceptualized as the intraorganismic and extraorganismic factors by which affect arousal is controlled, modulated, redirected, and modified to enable an individual to function adaptively in emotionally arousing situations, affect regulation is viewed as a central developmental task of the early months of life that possesses wide-ranging implications for children's development. Maltreated infants have been shown to display negative affects such as fear, anger, and sadness at earlier ages than do nonmaltreated infants (Izard et al. 1995; Sroufe 1996). Thus, it is conceivable that child maltreatment facilitates the premature development and closure of the negative affect pathways in the brain (Cicchetti and Toth 1998).

Corroboration for the belief that maltreated children are at risk for a developmental progression from affect regulatory problems to behavioral dysregulation has been obtained in a number of cross-sectional and longitudinal investigations (Cicchetti and Lynch 1995). Physically abused youngsters manifest the later vestiges of early affect-regulatory problems in the coping patterns they evidence when they are exposed to interadult anger, be it assessed directly through witnessing an angry simulated live laboratory interaction directed at their mothers (Cummings et al. 1994) or via observations of videotaped segments of adults engaging in unresolved and resolved angry and friendly interactions (Hennessy et al. 1994).

In addition, some of the coping skills that maltreated children use are reflected in the types of cognitive control functioning that they employ in the service of affect regulation. For example, Rieder and Cicchetti (1989) found that maltreated children were more hypervigilant to aggressive stimuli during cognitive control functioning tasks than were nonmaltreated children. Specifically, maltreated children recalled a greater number of distracting aggressive stimuli. The readier assimilation of the aggressive stimuli by the maltreated children resulted in their exhibiting less cognitive efficiency and more impaired task performances than the nonmaltreated children. It is conceivable that hypervigilance and ready assimilation of aggressive stimuli develop originally as an adaptive coping strategy in the maltreating environment, alerting the child to signs of imminent danger and keeping affects from rising so high that they would incapacitate the child. However, this response pattern may become less adaptive when the child is confronted with nonthreatening situations, and it may even undermine the child's ability to function adaptively under normal circumstances (Rogosch et al. 1995).

Although most of the research on affect regulation in maltreated children has been conducted at the behavioral level, there is increasing interest in its physiological correlates. Physiological adjustments to chronic stress, particularly when they occur early in development, may play a role in the behavioral and emotional sequelae of maltreatment. Physiological and behavioral responses to maltreatment are expected to be interrelated and to lead children to make choices and respond to experiences in ways that support pathological development (Cicchetti and Tucker 1994*a*).

Even though we possess limited empirical knowledge about the neurobiology of children who have grown up in a maltreating environment, information is accumulating on the functioning of the hypothalamic-pituitary-adrenocortical (HPA) system in maltreated children. Hart, Gunnar, and Cicchetti (1995) examined the salivary cortisol concentrations and social behavior (via repeated observations in the classroom, during outdoor activities, and through teacher reports) of maltreated and nonmaltreated children. The maltreated youngsters were studied while they attended a therapeutic preschool for abused and neglected children, and the nonmaltreated children were studied when they were enrolled in a preschool that served economically disadvantaged families. Each child's cortisol values over a number of weeks were used to compute measures of basal activity (median cortisol) and reactivity (ratio of quartile ranges). A child with a reactive HPA system would be expected to have a larger positive than negative quartile range.

Although median cortisol was not significantly correlated with social behavior measures, cortisol reactivity was positively correlated with social competence and negatively correlated with shy/internalizing behavior. Furthermore, maltreated children exhibited less cortisol reactivity than did comparison children. Maltreated children also scored lower in social competence and higher in shy/internalizing and acting out/externalizing behaviors. Maltreated children also failed to manifest elevations in cortisol on days of high versus low social conflict in the classroom. Social competence also was shown to correlate positively with cortisol levels on high conflict days. Taken in tandem, these results suggest a reduction in cortisol reactivity in maltreated children related to the impairment in social competence frequently noted among these children (see, for example, Kaufman and Cicchetti 1989).

In another study, Hart and colleagues (1996) investigated the effects of maltreatment on physiological and affective functioning in a group of maltreated children attending a summer daycamp. Maltreated children were found to have slightly elevated afternoon salivary cortisol concentrations, whereas their morning concentrations did not differ significantly from those of nonmaltreated children. Neither clinical levels of depression nor internalizing or externalizing problems were predictive of these elevated afternoon values. Depression among maltreated children was, however, associated with altered activity of the HPA system. Specifically, depressed maltreated children evidenced lower morning cortisol concentrations compared to nondepressed maltreated children and were more likely to show a rise rather than the expected decrease in cortisol from morning to afternoon. In addition, there was no evidence that depressed, nonmaltreated children exhibited this change in diurnal cortisol activity.

Yehuda and her colleagues (1991) have noted that the glucocorticoid response to aversive stimulation may help to normalize the increased activity of limbic midbrain structures in response to events that stimulate strong negative emotions. Thus, long-term dampening of the HPA system response to stressors may play a role in maltreated children's maladaptive affect regulatory capacities, reduced ability to engage in active avoidance in stressful circumstances, and increased likelihood of passive avoidance-like behaviors.

The Development of a Secure Attachment

The formation of a secure attachment relationship with the primary caregiver is considered the paramount developmental issue of the latter half of the first year of life. Ainsworth and Wittig (1969) identified individual differences in the quality and patterning of infant-caregiver attachment relationships (i.e., secure Type B, insecure-avoidant Type A, and insecure-resistant Type C) that are thought to reflect different styles of affect regulation that develop out of the children's history of distress remediation and emotional synchrony with their caregivers. Working models of attachment relationships are thought to develop through interactions with the primary caregiver. These models are considered to reflect a system of expectations about the caregiver's relative responsiveness and effectiveness in modulating and alleviating the child's physical and psychological needs, including the regulation of affect.

In contrast to normative samples, most maltreated infants develop more atypical insecure disorganized/disoriented (Type D) attachment relationships with their caregivers (Carlson et al. 1989). These attachments are characterized by a number of anomalous symptoms that are displayed in the presence of the primary caregiver, including dazed expressions, behavioral stilling and freezing, and apprehension (see Main and Solomon 1990). Frightened and/or frightening parental behavior are thought to be major etiological pathways to the development of Type D attachments (Main and Hesse 1990). It is conceivable that the early emergence of negative affects (e.g., fear, anger, sadness) in maltreated infants may predispose them to develop disorganized/disoriented attachments.

Maltreated preschoolers and school-aged youngsters continue to manifest a preponderance of insecure attachments, although there appears to be a reduction in the percentage exhibiting Type D attachments. Cicchetti and Barnett (1991) and Lynch and Cicchetti (1991) have shown that maltreated preschoolers and school-aged youngsters show an approximately 30–35 percent rate of atypical attachments.

Longitudinal studies reveal that securely attached maltreated youngsters are likely to become insecurely attached over time (Cicchetti and Barnett 1991; Schneider-Rosen et al. 1985) and that maltreated children who are insecurely attached to the primary caregiver are more likely to develop similar insecure patterns with other attachment figures (Howes and Segal 1993; Lynch and Cicchetti 1991). This concordance of insecurity across diverse attachment relationships suggests that the working models of maltreated children may be more aptly described as closed to incorporating information from more positive relationships with new persons. It appears that maltreated children form generalized models of attachment relationships based on their relationship history with their primary caregiver rather than specific models based on their individual history with a given person.

Thus, early stresses, either physiological or emotional, may condition or sensitize young neural networks to produce cascading effects through later development, possibly constraining the child's flexibility to adapt to new challenging situations with new strategies rather than with old conceptual and behavioral prototypes. There has been remarkable evidence that early psychological trauma may result not only in emotional sensitization, but also in pathological sensitization of neurophysiological reactivity (Cicchetti and Tucker 1994*a*; Perry et al. 1995). For example, Pollak and colleagues (1997) elicited event-related potentials (ERPs) from maltreated and nonmaltreated school-aged children. Children were presented with black and white photographs depicting posed facial expressions of anger, happiness, and neutrality. In each of two conditions, children were instructed to attend and respond to the angry or the happy face by pressing a button held in their preferred hand. The nonmaltreated children evinced comparable ERPs in both conditions. In contrast, maltreated children displayed lower ERP amplitudes to happy, as compared to angry, stimuli.

These findings are indicative of increased neurophysiological activity in maltreated children to angry affect and suggest increased psychological salience of negative affect, and possibly anger, for children with histories of maltreatment. In accounting for these findings, it was proposed that angry faces activated affective memories that were consistent with the mental representations of maltreated children. Indeed, such patterns of activation and memory would be adaptive for coping with environments marked by stress and threat. However, diminished responsiveness to positive affect combined with biases toward negative affect would certainly create difficulties for the children when interacting with others (e.g., peers, teachers) in nonmaltreating contexts (Rieder and Cicchetti 1989; Rogosch et al. 1995).

The Ontogenesis of Autonomous Self-System Processes

During the second half of the second year of life, children begin to develop a sense of themselves as autonomous agents (Emde et al. 1976). Prior to this period, the processes of affect regulation are largely sensorimotor in nature. However, with the acquisition of a sense of self, the child's development is characterized by a transition from sensorimotor to representational capacities (Sroufe 1996; Stern 1985). This transition period from infancy to toddlerhood is accompanied by concomitant reorganizations in the child's affect regulatory strategies. Thus, investigations of the development of the self-system are especially relevant to understanding the development of affect regulation, because they enable us to examine the role of growing representational capacities in the modulation of affect. The confluence of these cognitive, emotional, and representational reorganizations also bring about a natural change in the caregiver-child attachment relationship, because during this developmental transition, the burden of regulation shifts from caregiver to child (Emde et al. 1976; Sroufe 1996). Relatedly, during this period, children develop working models of themselves and of themselves in relation to others (Sroufe 1996).

A growing body of research reveals that maltreated children have grave difficulties with the successful development of these self-system component processes (Cicchetti 1991). For example, Schneider-Rosen and Cicchetti (1991) discovered that maltreated toddlers, in contrast to the positive affect exhibited by nonmaltreated youngsters, predominantly display negative affect upon recognizing themselves in a mirror-and-rouge task. Likewise, Beeghly and Cicchetti (1994) found that maltreated toddlers use less internal state (i.e., self) language, especially emotion words that refer to their physiological states and to negative affect. The self-language findings are congruent with those of Crittenden and DiLalla (1988), who reported that maltreated toddlers displayed an overbright affect and compulsively complied with the requests of their maltreating caregiver, both behaviors reflective of adapting a "false self" to escape further abuse.

Using a narrative story stem completion task, Toth and coworkers (1997) investigated preschool-aged maltreated youngsters' maternal representations, self-representations, and relationships with an examiner. Maltreated children differed substantially from nonmaltreated children in terms of their maternal and self-representations. Specifically, maltreated preschoolers had more negative representations of maternal figures and of themselves; moreover, as would be expected based on these negative representations, maltreated children also were more controlling with and less responsive to the examiner.

However, these differences between maltreated and nonmaltreated children obfuscate some of the more complex differences found among subtypes of maltreated children. Physically abused youngsters evidenced the most negative maternal representations and also had more negative self-representations than did nonmaltreated children. Interestingly, sexually abused children manifested more positive self-representations than neglected children. Despite these differences in the nature of maternal and self-representations, physically and sexually abused children both had less positive relationships with the examiner, evidencing more controllingness and less responsivity. This investigation adds to the corpus of knowledge regarding disturbances in the self-system functioning of maltreated children and provides support for relations between working models of self and other and the self-organizing function that these models exert on children's lives.

Further insight into the organization of self-system processes in maltreated children is provided by investigations on the development of perceived self-competence in maltreated children. During the preschool and early school years, maltreated children show a self-enhancement bias over and above that normally displayed by nonmaltreated children throughout this age range. Beginning at approximately 8 to 9 years of age, when social comparison processes become more sophisticated and the self-system consolidates, maltreated children display more negative self-evaluations than do nonmaltreated children. Moreover, the negative self-evaluations of maltreated children actually underrate their true competence, at least as assessed by teacher ratings (Vondra et al. 1989, 1990). The lability of self displayed by maltreated children (i.e., grandiose early, negative late) may portend, or at least be a prospective link to, the development of personality disorders (e.g., borderline personality, dissociative disorder) commonly found in studies of adults who retrospectively report having been maltreated in childhood.

Resilience in Maltreated Children

The notion of an average expectable environment for species-typical development suggests that competent outcomes in maltreated children are highly improbable because of wide-ranging disturbances in the maltreatment ecology (Cicchetti and Lynch 1995). However, although there is documented risk for maladaptation associated with maltreatment, the absence of an average expectable environment does not condemn maltreated children to negative developmental outcomes later in life. Despite the relatively low probability of adaptive outcomes for maltreated children (in comparison to nonmaltreated children), individuals' self-righting tendencies (Waddington 1942, 1957), in combination with the presence of any additional intraorganismic as well as extraorganismic protective mechanisms and compensatory or protective factors, may result in some maltreated children displaying developmental plasticity and achieving competent functioning.

In an investigation of resilience in school-aged children, maltreated children as a group exhibited lower overall competence across multiple areas of adaptation than did nonmaltreated impoverished children (Cicchetti et al. 1993). However, whereas more maltreated children than nonmaltreated children exhibited a low number of indices of competence (i.e., 0 or 1 out of 7), an equal proportion of maltreated and nonmaltreated children demonstrated a moderate to high number of indices of competence.

More specifically, ego-resilience, ego-overcontrol, and positive self-esteem each accounted for significant amounts of variance in the adaptive functioning of maltreated children. In contrast, only ego-resilience and positive self-esteem contributed unique variance to account for adaptation in nonmaltreated children. A reserved, controlled approach to the environment may help maltrreated children to be more attuned to adapting to the adverse conditions of their home environments and may protect them from being targets of continued maltreatment incidents (Crittenden and DiLalla 1988). Clearly, pulling back from conflict in the family, detaching from high-intensity affect, and being compliant with the wishes of one's caregiver all can help one to escape abuse and/or to achieve competent adaptation (cf. Werner and Smith 1992).

Conclusion

The findings presented in this chapter make clear that further examination of maltreated children's development and struggles with adaptation will teach us about the range and variability of individual response to challenge and adversity and help to specify the limits of biological and behavioral plasticity. Conceivably, during so-called transitional turning points or sensitive periods in development, resilient individuals may maintain the ability to use self-righting tendencies when their higher level monitors detect deviances in one or more biological and behavioral subsystems. In contrast, some children (e.g., the least competently functioning ones) may reveal a total absence of resilient self-strivings, suggesting that their individual neural and psychological self-organizations are in disarray. Although we are in the early phases of our research on the processes contributing to resilience in maltreated children, further investigations of this population will contribute greatly to our understanding of developmental plasticity through the elucidation of the mechanisms and processes that lead to adaptive versus maladaptive outcomes.

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Commentary

Concepts and Metaphors for a Multidisciplinary Approach to Developmental Plasticity

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Becoming Multilingual

The talks we have heard this morning truly exemplify the language problem that Dr. Kupfer referred to in his Keynote address. They represent the diverse disciplines studying developmental plasticity, each at a different level of analysis. The speakers used terms with which we are all familiar and could even define, if we had a few moments to pause and think carefully. Yet the terms have been spoken rapid-fire by native speakers, who are not focusing on the terms, but using them to make larger conceptual points. Our tenuous grasp of the terminology hampers our understanding of the speakers' concepts. Thus, one of our goals is to acquire a working, conversant knowledge of all of these languages so that we can appreciate the common ideas underlying the different disciplines.

This goal is truly staggering. For example, it struck me that the following words are absolutely commonplace within each discipline, and yet those of us in allied disciplines do not have a facile working knowledge of them: "synaptogenesis," "receptor ligand and agonist," "variable decelerations," "behavioral state," "insecure attachment," and "ego resilience." These are all very standard terms in each of their disciplines. However, many have technical meanings with nuances achieved after long debate within the field, nuances that are quite different from the common usage of the words.

This language problem makes it difficult to transcend boundaries among the multiplicity of disciplines. I am not offering a solution, but marking a problem

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with which we are all struggling as we sit here. What we are trying to do at this conference is to become conversant in each of these languages, to become multilingual, so that we can identify and integrate the ideas common to diverse disciplines.

Biased Metaphors for Development

It is absolutely clear that we all recognize that developmental plasticity results from a reciprocal interaction between biology and the environment. Nonetheless, most speakers used the traditional metaphors for development even when their data actually demonstrate that these metaphors are not accurate. The traditional metaphors are biased toward biological determinism and inadequately represent the role of the environment in development.

One of the reasons that traditional metaphors are biased is that they arise from the stunning phenomenon, the miracle, of growth—the fact that a complex organism, such as a human being, does indeed develop from a single cell. To emphasize this singular origin, traditional metaphors have been "a blueprint for development," "genetic switches," "developmental trajectories," and "canalization."

A focus on DNA has given rise to metaphors such as the "blueprint" for development—the idea that the organism contains genomic information that needs simply to be read out by the environment. This metaphor implies that the genome has most of the information for the development of a structure. For example, the SRY gene that Bruce McEwen talked about is often touted as "the" gene for maleness. But we know that the gene actually is à trigger for the development of the testes and, ultimately, testosterone production. Testosterone, in turn, becomes the environment for other developing cells and determines which of their genes will be expressed.

Even when we acknowledge that maleness is not a single-gene trait, that the SRY gene does not contain the whole plan for maleness, this gene is still talked about as a switch. This is a "railroad track switch" metaphor—implying that there are two or more existing pathways and that the gene throws a switch to determine the path of development. But notice that, in the switch metaphor, it is the locomotive and the inertia of the train that takes the organism down its predetermined track. Other than throwing one switch, the surrounding environment has virtually no role in determining where the train goes.

A similar metaphor is a "trajectory"—the idea that the developing organism is launched. In this metaphor, environmental events can change the developmental trajectory. Still, the general direction and progression come from within the organism—its booster rockets launch it into a barren environment with only microgravity. Thus, the role of the environment ranges from small midcourse corrections (causing the organism to be "on target" or "off target") to extreme perturbations that cause a crash and burn from which there can be no recovery. Notice also that the trajectory metaphor has the implicit idea that there is a target in development; there is optimal final form toward which development is aimed.

More sophisticated models, like Waddington's epigenetic landscape, use the "canalization" metaphor. Here the pathway of development is at least downhill, not up against gravity. Nonetheless, the main force of development is singular, the gravitational field. While the landscape (environment) is varied, it merely canalizes or channels the developing organism down one route or another. The environment, like a mountain, is fixed, and if the organism encounters enough small bumps and dips to be diverted over a ridge and down its far side, then there is no way to get back. The environment is static and cannot respond to the developing organism, enabling it to get back over the ridge and down into its home valley.

Obviously, these traditional metaphors are extremely useful and have served us for a long time. They are all good metaphors for what I am calling "upward causation," the process by which genes, cellular interactions, and intrinsic physiology cause development at the behavioral level, similar to the way these molecular and cellular mechanisms cause development of anatomical structure.

Downward Causation

It is striking that all of this morning's presentations demonstrated that this traditional focus on upward causation is not sufficient for understanding development. They each made this point implicitly, if not explicitly. Each one not only emphasized the importance of the environment, but provided many examples of what I call "downward causation"—the causal process in which events at higher levels of analysis actually regulate events at lower levels. Recognizing the importance of downward causality enables us to view the developing organism as a reciprocally interacting system and not a unidirectional process that can be reduced to, that is, simply explained in terms of, genetic and cellular events. This reduction is not feasible, either in practice or in principle.

This is an essential conceptual advance, and one made by each of the speakers today. Dr. Cicchetti showed very elegantly how chronic, unresolved anger in a family can affect psychological coping mechanisms, such as hypervigilance in an individual. In turn, this cognitive process may well be mediated through the tone of the amygdala, what McEwen called "the ganglion of fear." Moreover, in addition to emotional consequences such as increased anger, this psychological coping mechanism has physiological consequences, such as increased adrenal reactivity.

Dr. Emory talked about birth, emphasizing that this transition, made by all mammals, entails the intense stimulation of the delivery process as well as emergence from the relatively constant environment of the womb into a cacophony of novel environmental stimuli. During birth, the baby experiences as stressors first the uterine contractions, the mechanics of squeezing through the birth canal, and finally the sudden onset of external stimulation. This massive environmental input triggers physiological responses that are actually necessary for development of a wide variety of systems, ranging from anatomy, for example, lung development, to such psychologically sophisticated concepts as temperament and personality.

Dr. McEwen talked about response to stressors and maternal care. His work is particularly striking because here, too, a social system, the parental interaction, regulates a physiological system, the hypothalamic-pituitary-adrenal axis. Particularly interesting was his demonstration that downward causation operates in several different timeframes across the lifespan. He elucidated how adrenal reactivity can be altered early in development, how this alteration is manifest later, not only during adulthood but also during aging and, framed more positively, improves resistance to disease and insult later in the lifespan.

Dr. Constantine-Paton talked about the effect of visual stimulation on neural development that occurs when the eyes first open. This developmental milestone is not achieved in rats until well after birth. At approximately 2 weeks of age, the eyes open, and light from the environment has a critical role in the developing visual system; it turns off synapse formation in the superior colliculus. This experience and the anatomical changes it produces are necessary for developing the inhibitory systems that will end the critical period for neural structure development in the visual system. They also mark the beginning of the period when specific sensory stimuli lead to the development of a specialized nervous system capable of processing specific visual information.

The Organism as Stone or Clay

Having established that downward causation is commonplace and operates from the social level of analysis down to individual behavior and further down to regulation of genetic expression in developing cells, we now need to understand the functions that downward causation serve in development. In order to illuminate this issue, I suggest we use different metaphors for development, those of sculpture and ceramics. These metaphors are very different from those discussed earlier. Here the basic biological substrate is simply a stone or a lump of clay, and the impetus for change and development comes from the environment, not the organism. In neuroscience, it is common to talk of environmental input as "sculpting" the nervous system by removing excess and extraneous neural structures and leaving those connections that are essential for a particular function.

In other systems, the developmental process may be more like creating a porcelain pitcher from a lump of clay. Not only must excess material be removed, but the remaining clay must also be pulled, thinned, and shaped, and finally the handle must be added. This developmental process is achieved only by the simultaneous interaction of many external forces operating on the clay: the hands of the potter, of course, but also the centripetal force of the spinning wheel, the weight of the wheel that gives it its inertia, the potter's kicking foot which controls that inertia, and finally, gravity and even the humidity of the room.

Functions of Downward Causation

One of the functions of downward causation and environmental change is to create the basic conditions for change, similar to the forces that maintain the spinning of the ceramist's wheel. Constantine-Paton's work is an excellent example of the nervous system's basic activity as necessary impetus for development, with anatomical structure and energy for metabolism as environmental inputs.

Constantine-Paton demonstrated how a "map" of the retina is created in superior colliculus; it has a retinotopic architecture that elegantly parallels the location of receptors on the retina. The spontaneous activity of receptors mutually reinforces adjacent receptors because they are close enough to be reciprocally affected. There is a basic inertia to the system; spontaneous firing that is environmentally maintained by the input of glucose and oxygen. This reinforces spontaneous activity, before the eyes open and without visual stimulation.

The specific environmental input is simply the fact that two receptors in the retina are next to each other. Located in the same area of the retina, the cells are more likely to affect each other and thus fire together in space and time. This mutual stimulation leads to the selective maintenance of the projections to the superior colliculus that come from adjacent places in the retina. Spontaneous activity from places in the retina that are far apart do not mutually reinforce each other. In this way, the retinotopic mapping in the superior colliculus—its spatial representation of the retina—becomes possible.

Another function of downward causation is signaling the context for change, providing environmental information that signals a change in the functional requirements faced by the individual and using this common signal to enable coordinated development among many different systems. This was Dr. Emory's point about the birth process. It is a signal for a change from endogenous drive to exogenous drive. For example, Emory described how uterine contractions lead to the differentiation of lung maturation, perhaps by the expelling of mucus. In other words, the "stress" of labor has a positive effect and is indeed required for normal maturation. Moreover, he presented evidence that the birth process, with its uterine contractions and changes in oxygenation that have profound neuroendocrine effects, may even have a causal effect on such complex behavioral traits as temperament. Obviously, we do not yet know whether the association between the nature of the birth process and temperament is causal or correlational (we are right at that cusp of trying to tease this out), but it is an important idea that a primary function of the environment is signaling the context for qualitative change during development.

The third function of downward causation during development is to provide input that is absolutely necessary for the development of a specific function, a specific size, or a specific form that is needed for adaptive or mature function. This brings us back to the ceramics metaphor—the role of the hands in shaping the emerging pitcher, creating a global cylinder on the wheel and pinching the depression at the edge for the spout. In this type of development, the environment has specific information requisite for a specific form and function.

Martha Constantine-Paton referred to the research on binocular vision demonstrating that both eyes must see and convey environmental information in order to create the neural system producing binocular vision. This type of development is what Ernst Mayr called an "open program"; normal functional development cannot occur without the required environmental information.

Another excellent example was provided by Bruce McEwen's work on the essential role of maternal care in the development of a functional adrenal response in adulthood. He described how specific stimulation during maternal care shapes the birth and death of cells in the dentate gyrus, which regulates ultimately that ganglion of fear, the amygdala. In turn, Cicchetti told us, this brain region becomes crucial in adulthood for determining whether a given environmental stimulus is perceived as a positive or negative emotional expression and whether it triggers a fear response, an anger response, or positive empathy.

Downward causation is also the conduit for environmental disruption and the creation of pathology. Dr. Cicchetti has given some absolutely stunning examples of the effects of sexual abuse and unresolved anger decreasing reactivity and altering the image of a caretaker. In this form of pathology, the effect of a disruptive environment is mediated through a variety of different psychological constructs like cognition, attention, and affective tone.

Finally, we also need to extend and expand these concepts into the normal domain. In the course of normal development of healthy resilient functioning, there are undoubtedly beliefs, social interactions, and properties of the individual and of the social structure that act as causal agents in regulating specific biological and cellular mechanisms of development.

Types of Clay: Is There a Common Substrate?

Are the mechanisms mediating these different types of downward causation the same? Is there a common substrate for development? This is asking about common and distinctive properties of different types of clay. For example, is there a common element in the biological structure of the brain that is being shaped during development of neural systems that serve diverse functions?

Martha Constantine-Paton and Bruce McEwen gave some evidence that the answer can be yes, at least in some systems. The NMDA receptor was a fundamental unit in both of their talks. This receptor needs depolarization to work, and it is an important part of synaptogenesis. So, it is an important part of the refinement of the specific connections in the retina and in the amygdala. In addition, it is an important part of estradiol effects on sexual differentiation of the brain. As Bill Greenough told us from the audience, it plays a pivotal role in the long-term potentiation effects of learning seen in layers 2 and 3 of the neocortex. Taken together, these results suggest that there may be some common cellular mechanisms that mediate all these different types of downward causation.

On the other hand, the answer may well be no; there is not just a single or very limited set of mechanisms or developmental substrates. This conclusion is suggested by the nature of development itself. How does an organism maintain stability when it is itself developing and changing? How does it maintain stability in a changing environment? What kind of energy and what kinds of forces are interacting to achieve this goal? For example, stress and adrenal function have been mentioned in most of the talks this morning. Traditionally, the focus has been on the hypothalamic-pituitary-adrenal axis and the role of corticosterone in neural function, particularly in the context of producing homeostasis—adrenal function within normal parameters.

But the adrenal may also work along with other systems to provide the energy and information to maintain constancy in the face of change and external challenges. Bruce McEwen has proposed the term "allostasis" to refer to this role of adrenal function.

Allostasis is not a substitute for the stress response. It is another equally important role played by the adrenals in a related but distinct functional context, namely, the energy required to maintain homeostasis. As an example, I like to use what goes on in an animal care facility. The Federal standard is homeostatic; for rats, the room temperature must be maintained at 70±2 °F. Allostasis refers to the fact that achieving this goal costs much more in Minneapolis or Tucson than it would in Hawaii, where the outside temperature is almost always closer to the Federal mandate. In Minneapolis, one must pay to warm the air two-thirds of the year. In Tucson, one has to pay not only to cool the air, but in order to tightly control the temperature without wide fluctuation, it must be overcooled first and then reheated.

Thus, in order for the adrenal to provide allostasis, its functioning must be coordinated with an entirely different constellation of events than if we were just to focus on homeostasis. We should, for example, consider the basic substrate required for the immune system, for energy and metabolism and for cardiovascular change. In this case, there cannot be a single substrate, because so many different physiological systems are involved.

I would like to suggest that this concept is important not only for diverse systems interacting at the physiological level but also for those interacting at the psychological level. For example, Dr. Cicchetti talked about regulation of affect. What is the effect in a child who must maintain attachment to a caregiver in the face of physical abuse? This has to have an allostatic load different from maintaining attachment to a supportive and nurturing caregiver. One aspect of this higher load is stress response mediated by the adrenals; another is a decrease in emotional reactivity, mediated by cognitive processes. The consequence of having to bear that allostatic load during development is more anger, fear, and aggression, rather than the more appropriate responses of empathy and concern.

Overview

In summary, each talk this morning provided exemplars of the functions of downward causation operating across all levels of analysis, ranging from social interactions and culturally based beliefs to regulation of specific genes. It is our goal to specifically identify the reciprocal interactions among genes, the nervous system, hormones, behavior, and the social environment which, taken together, define developmental plasticity.

Cognition

Cellular and Molecular Mechanisms of Cortical Development: Relevance to Congenital Cortical Malformations

Pasko Rakic

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ABSTRACT

The laminar, modular, and areal organization of adult cerebral cortex is the end result of a series of cellular and molecular events that occur during embryonic development. Individual neurons are generated at a specific time and place in the proliferative zones, and then they become allocated to final positions in the cortex, migrating along specific routes while acquiring distinct phenotypes. These early developmental events are essential for the establishment of normal species-specific cortical cytoarchitecture, synaptic connectivity, and function. To analyze the roles of cell lineage and cell-cell interactions in allocation of postmitotic cells in the primate cortex, we use recombinant retroviruses to label individual progenitor cells in the proliferative zones and then follow the migration of their progeny as they assume their final positions.

The results show the relationship between the type of cell division and the mode of cell allocation. Neuronal clones resulting from the symmetrical divisions become distributed in horizontal arrays, while the asymmetrical cell divisions, which become more prominent during the course of corticogenesis, produce cohorts of neurons that retain a radial alignment, even in the convoluted primate cerebrum. This complex morphogenetic process requires orchestration of multiple molecular events, including selection of a migratory pathway that is provided by heterotypic membrane-bound cell recognition receptors, binding of cellular substrates

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through a variety of homotypic adhesion molecules, and activation of specific ion channels/receptors that provide second-messenger- mediated signals to the cellular machinery involved in motility.

To examine the role of individual molecular components, we generated polyclonal antiserum (D4) and monoclonal antibody (NJPA1) that recognize plasmalemmal junctions between migrating neurons and adjacent radial glial fibers during the phase of cell migration. The addition of D4 and NJIA1 to the culture medium in both imprint and slice preparations leads to the withdrawal of the leading process, changes in microtubular organization, cessation of movement, and finally, detachment of neurons from the radial glial shafts.

Using an acute slice preparation in conjunction with calcium indicator dyes, we show that a combination of selective voltage- and ligand-activated ion channels cooperatively controls calcium influx into the migrating cells and, thereby, regulates their cytoskeletal composition, thus determining polarity and maintaining the rate of cell movement essential for their proper placement.

Together, these results suggest that early communications between heterogeneous classes of cells play a major role in the selection of migratory pathways as well as in the restriction of cell fates before they arrive at their final destination and form synaptic connections. After the basic axonal pathways of the cerebral cortex have been laid down, survival of individual connections and fine tuning of initially overproduced synaptic terminals become sculpted by functional validation, which in primates lasts until sexual maturity. Thus, extrinsic factors provide for the individual variations of synaptoarchitecture within the constraints of primordial cortical organization established during embryogenesis. New experimental approaches allow examination of the role of specific molecular components that mediate early developmental events and provide insight into the pathogenesis of acquired and genetic disorders of the cerebral cortex that underlie a range of cognitive disorders.

Propagation of Developmental Errors Accounts for Perceptual and Cognitive Deficits in Learning Disability

Albert M. Galaburda

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ABSTRACT

I am struck by arguments regarding mechanisms of developmental learning disorders that take a remarkably phrenological position. Thus, in dyslexia research, debate rages about whether the condition reflects disordered cognition or disordered perception, implicating higher or lower brain centers, respectively. This debate completely ignores what is already known about plasticity in the developing brain and cannot be supported by our own research involving animal models of learning disorders. It is not likely that early acquired lesions injure only one area, but rather trigger a series of changes that propagate along pathways from processing station to processing station. It is also possible that this propagation, in addition, makes use of corrections that are usually transient during development, thus permitting the emergence of unusual processing architectures, with possibly unusual behavioral characteristics.

Ten years of research in our laboratory shows that the most likely first event in the dyslexic brain is the formation of multifocal minor cortical anomalies of neuronal migration. These tend to affect the perisylvian cortex–mainly the inferior prefrontal and premotor regions. At the same time, we have shown changes in cell packing density and cell size in thalamic relay nuclei such as the medial geniculate nucleus and the lateral geniculate nucleus. We propose that the thalamic changes represent plastic propagation of the frontal lobe changes along correctional pathways.

In order to test this hypothesis, we induced minor malformations attributed to altered neuronal migration in the frontal, parietal, and occipital cortex of newborn rats. Analysis of thalamic nuclei showed changes in cell sizes comparable to those demonstrated in the human

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dyslexic brain. Connectional studies using DiI and other tracers also showed that patterns of connectivity are substantially changed in the presence of these small and focal malformations.

We have initiated a study of another, possibly developmental, disorder—schizophrenia. In this condition, frontal lobe anatomy has been reported to be altered. Preliminary findings show that connectionally related areas in the temporal lobe, too, show evidence of altered cell packing density and cell size in this disorder. We propose that the lower level functional anomalies reported in dyslexia, and possibly some of the perceptual complaints associated with schizophrenia, may reflect the propagation of changes across processing stations, via connectional pathways, arising in distant brain regions during early brain development.

Neural Plasticity in Humans: Evidence From Children With Early Focal Brain Injury

Elizabeth Bates

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ABSTRACT

The nature-nurture controversy has been with us for a long time, but some of the strongest claims about the innate knowledge in humans have come from 20th century linguistics. Since the late 1950s, the MIT linguist Noam Chomsky has argued that human language is a kind of mental organ; general principles of learning and development have no more implications for language than they have for the growth of an arm or a kidney. Philosopher Jerry Fodor has taken this hypothesis one step further, arguing that language is just one of many mental organs or "modules," special-purpose processors that have emerged in our species to handle categories of information that are (1) unique to humans and (2) so special that they cannot be learned by any general-purpose learning device, and (3) each has its own dedicated neural architecture and (4) its own unique maturational course.

This vision of biology and the mind has a long history (dating back to Gall and Spurzheim's theory of phrenology), and it was consistent with what we knew about the brain for the first half of this century. However, the last 20 years of research in developmental neurobiology have raised serious problems for the nativist view, suggesting instead that the primate brain is extraordinarily plastic, and that cortical specialization is largely determined by brain activity and experience.

This presentation reviews results from a large project devoted to the study of linguistic, cognitive, and affective development in children with prenatal or perinatal injuries to the left or right hemisphere. These studies lead to the following conclusions: (1) recovery from focal brain injury is indeed far better in young children than it is in adults with similar injuries; (2) there are specific effects of lesion site in all these areas of development,

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suggesting some degree of localization from the very beginning; (3) the mappings between lesion site and behavioral outcomes are qualitatively similar to those observed in adults within the areas of spatial cognition and emotional expression, but (4) the mappings between lesion site and language are entirely different for children than they are for adults with analogous injuries.

Although there is plasticity in all these areas of development, human language actually shows more plasticity and more variation than any other domain that we have studied. This may be due to the phylogenetic recency of language, but it may also reflect the highly distributed nature of linguistic representations (i.e., the whole brain is involved in meaning, and for that reason, the whole brain is involved in language), and the arbitrary nature of the mapping between meaning and sound (i.e., language does not provide natural selection with the kinds of "physical constants" that are required to instantiate highly specified innate outcomes).

Behavioral Neurogenetics Research: A Method for Analyzing Linkages Among Gene, Brain, and Behavior

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**Stanford University and* †*Johns Hopkins University*

Several decades of research on behaviorally defined syndromes such as autism, ADHD (attention deficit hyperactivity disorder), mental retardation, and learning disabilities suggest that rapid progress toward understanding underlying genetic and neurobiological factors may be impeded by the etiological heterogeneity of individuals meeting the widely accepted diagnostic criteria that define these important disorders. Continued research aimed at further subdividing these behaviorally defined syndromes into etiologically meaningful subgroups is essential to our eventual understanding of the pathogenesis of childhood cognitive and behavioral disorders.

However, a complementary research strategy that has been promoted at the Behavioral Neurogenetics and Neuroimaging Research Center (BNNRC) of the Kennedy Krieger Institute/Johns Hopkins focuses on multilevel scientific study of individuals with known or suspected homogenous genetic etiology for cognitive, behavioral, and developmental dysfunction. This approach is similar to more traditional neuroanatomically based lesion studies except that the "lesion" begins at the DNA level. Accordingly, we have coined the term "behavioral neurogenetics" to represent this new research approach.

Two important assumptions underlie behavioral neurogenetics research. The first is that the complex pathway beginning with one or more genetic factors affecting brain development or function, and ultimately leading to behavioral or cognitive dysfunction, will be more accessible when studied with genetically homogeneous groups. The second assumption is that the information derived from the investigation of these "prototype" genetic conditions will be relevant to our understanding of brain-behavior associations in individuals with similar patterns of cognitive, behavioral, and developmental dysfunction from the general population. Despite the fact that behavioral neurogenetics research is a

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direct outgrowth of the relatively recent explosion in genetic knowledge, the research findings from the BNNRC and other centers already support the first of these assumptions.

In this chapter, our group's research on a specific genetic condition known as the fragile X syndrome is used to demonstrate the concept of behavioral neurogenetics research. Data obtained from molecular genetic, neuroimaging, cognitive, and behavioral investigation of individuals with the fragile X mutation illustrate how this line of investigation can help to develop an initial understanding of complex linkages among genetic, neuroanatomic, and behavioral variables. Mapping fundamental molecular events to specific neurobiological correlates and phenotypic features opens the possibility of establishing direct links between genetic etiology and cognitive and behavioral outcome. (For the remainder of this chapter, the term "neurobehavioral" is used in place of "cognitive and behavioral.")

Fragile X Syndrome and Research at the BNNRC

In approximately 20 years, knowledge of the most common known heritable cause of neurobehavioral disability has progressed from a relatively nonspecific clinical description to identification of the responsible gene and insights regarding probable protein function (Kersten and Stephen 1995). The fragile X syndrome, caused by mutations in the *FMR1* gene, is found in all geographic regions, ethnicities, and socioeconomic strata. Both genders are affected, the more severe male phenotype resulting from absence of the *FMR1* protein.

While qualitatively similar to the male phenotype, females show a broader range in severity of neurobehavioral features, likely reflecting varying protein levels in the brain. Given a population frequency of approximately 1 in 1,200 to 1 in 2000 and the presence of significant neurobehavioral dysfunction in most affected individuals, fragile X syndrome has important public health implications.

Our research group has spent more than 10 years engaged in research designed to elucidate the behavioral and cognitive features associated with fragile X. We also have focused considerable effort on using quantitative imaging methodology to identify brain regions that are affected by mutations of the *FMR1* gene. These studies have been successful in establishing a preliminary neuroanatomical phenotype associated with fragile X syndrome.

Recent studies aimed at delineating the profile of *FMR1* gene expression in the developing and adult mammalian brain provide strong support for the validity of this anatomical phenotype (Abitbol et al. 1993; Hergersberg et al. 1995; Hinds et al. 1993). These quantitative imaging data are also supported by the known or hypothesized involvement of these brain regions in neurofunctional circuits underlying behavioral and cognitive processes recognized as abnormal in individuals with fragile X syndrome (Abrams and Reiss 1995*a,b*).

A third focus of our clinical research laboratory has been the development and utilization of measures of *FMR1* gene activation (Abrams et al. 1994; Reiss, Freund, et al. 1995). Attempts to understand how these measures might be correlated with neurobehavioral function and brain anatomy have revealed potential pathogenetic mechanisms which might lead to neurobehavioral disability in the fragile X syndrome (Reiss, Abrams, et al. 1995). These studies suggest that the *FMR1* gene has its greatest impact on specific domains of brain function while leaving other domains relatively spared.

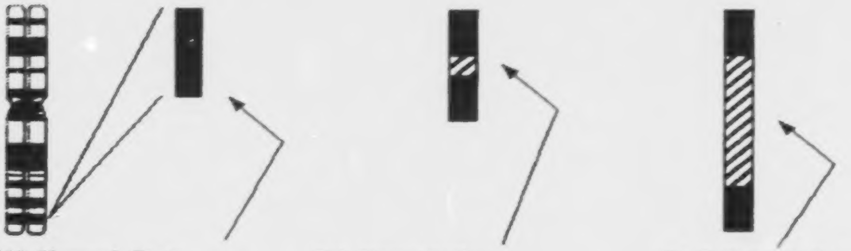
The long-term thematic mission of the BNNRC is to collect, analyze, and interpret scientific information from multiple fundamental levels of inquiry. We believe that this work will contribute to our understanding of the complex interplay among gene state, brain function, and environmental influences in the fragile X syndrome. Study of this important single gene condition will also contribute to our fundamental knowledge of linkages among gene, environment, brain, and behavior in children from the general population.

The Genetics of Fragile X Syndrome

Fragile X syndrome is caused by mutations in the *FMR1* gene located on the long arm (q27.3 band) of the X chromosome. Although the precise function of the *FMR1* protein (FMRP) is not yet known, recent evidence suggests a neuronal dendritic location and a possible role in regulating cellular translational activities through binding to the cytoplasmic ribosomal complex (Khandjian et al. 1996; Witt et al. 1995).

The most common mutation occurring at the *FMR1* locus involves expansion of a CGG trinucleotide repeat sequence within the promoter region of the gene (Verkerk et al. 1991). The number of *FMR1* CGG repeats present in unaffected individuals from the general population ranges from approximately 6 to 54 (Fu et al. 1991). When approximately ≥200 CGG repeats are present, the expanded repeat sequence and an adjacent CpG island are usually hypermethylated, a phenomenon associated with transcriptional silencing of the gene, absence of FMRP, and the occurrence of clinical manifestations of the fragile X syndrome (Hansen et al. 1992; Pai et al. 1994; Pieretti et al. 1991). This molecular state is commonly referred to as the *FMR1 full mutation* (figure 1). The intermediate range of repeats (approximately 50 to 200 CGGs) is referred to as the *premutation* and is characterized by the absence of methylation within the promoter region, production of FMRP, and presumed normal phenotype (Reiss et al. 1993; Rousseau et al. 1994).

Due to early postconception mitotic instability at the repeat locus, individuals having an expanded *FMR1* gene can have alleles of varying size (Wohrle et al. 1993). Some individuals have a combination of methylated and unmethylated alleles of differing size (i.e., falling within both the full mutation and premutation categories) and are referred to as having mosaic status for the *FMR1* gene. A small number of individuals have also been described as having



(A) Normal State
Schematic drawing of an X chromosome and normal size of the *FMR1* gene at the fra X locus. Adjacent CpG island is unmethylated (except on normal female inactive X).

(B) Premutation
In the Premutation, there is a small insertion of additional DNA in the *FMR1* gene (+50-600 bp). Adjacent CpG island is unmethylated (except on normal female inactive X).

(C) Full Mutation
In the Full Mutation, a much larger insertion is present in the *FMR1* gene (≥600bp). Adjacent CpG island is methylated.

Figure 1

large (i.e., >600 repeats) repeat lengths which may be partially unmethylated (Hagerman et al. 1994). This state is referred to as methylation mosaicism.

The molecular characteristics of the *FMR1* full mutation permit both gene activation (methylation) status and the size of the *FMR1* trinucleotide repeat expansion to be directly measured and quantified with computer-assisted methods (Abrams et al. 1994; Reiss, Abrams, et al. 1995; Reiss, Freund, et al. 1995). Recently, methods for quantitative assessment of *FMR1* protein levels have become available. Consequently, the contribution of the *FMR1* mutation to abnormalities of cognition, behavior, and brain morphology can be more directly examined.

A Neurobehavioral Phenotype

Most studies comparing males and females with fragile X syndrome to controls matched for age, gender, and IQ indicate a characteristic set of maladaptive behaviors associated with fragile X, including hyperarousal, social anxiety, and attentional dysfunction (Baumgardner et al. 1995; Cohen 1995; Freund et al. 1995; Bregman et al. 1988; Cohen et al. 1988, 1989, 1991; Hagerman 1991; Reiss and Freund 1992; Sudhalter et al. 1990).

Compared to both population norms and matched control groups, young girls with fragile X also exhibit maladaptive behaviors including problems with depression, social withdrawal, and hyperactivity (Freund et al. 1993; Lachiewicz 1992; Lachiewicz and Dawson 1994). Adult women with fragile X experience a high incidence of depression, abnormalities in social interaction and communication (Freund et al. 1992), and often meet many of the criteria for schizotypal personality disorder (Reiss et al. 1988: Sobesky et al. 1994).

The cognitive component of the neurobehavioral phenotype associated with fragile X has a characteristic profile of intellectual strengths and weaknesses.

This profile is quite similar for both genders, even though females with the full mutation typically function at a much higher overall intellectual level and manifest less severe deficits than males with the full mutation. The cognitive profile associated with the full mutation includes deficits in visual memory and perception, mental manipulation of visual-spatial relationships among objects, visual-motor coordination, processing of sequential information, and executive aspects of attention (Freund et al. 1995; Freund and Reiss 1991; Grigsby et al. 1987; Hinton et al. 1992; Kovar et al. 1993; Theobald et al. 1987). In contrast, studies assess- ing verbal-based skills in individuals with fragile X show both males and females to have relative strengths or spared abilities in this cognitive domain (e.g, Chudley et al. 1983; Freund et al. 1995; Freund and Reiss 1991; Grigsby et al. 1987; Hinton et al. 1992; Mazzocco et al. 1993; Theobald et al. 1987).

Supporting the presumption of a consistent cross-gender profile are recent data from our group, as well as others, suggesting that the profile of neurobehavioral features associated with this condition is largely unrelated to overall cognitive level (Mazzocco et al. 1993, 1995) and is evident in males by at least the age of 3–5 years (Freund et al. 1995) and in girls by early school age.

Neuroimaging

A necessary component of behavioral neurogenetics research is the investigation of neurobiological functions that are affected by the genetic factors of interest and that underlie the neurobehavioral abnormalities observed in affected individuals. Preliminary findings from magnetic resonance imaging (MRI) studies carried out in our laboratory suggest an association between the *FMR1* mutation and abnormalities of particular neuroanatomical regions.

Compared to matched controls, individuals with fragile X demonstrate morphological abnormalities of the cerebellar vermis, fourth and lateral ventricles, hippocampus, amygdala, and caudate nucleus (Reiss, Freund, et al. 1991, 1995; Reiss Abrams, et al. 1995; Reiss, Aylward, et al. 1991; Reiss et al. 1994). Putative dysfunction of these same brain regions in affected individuals is supported by findings from neurobehavioral studies (Abrams and Reiss 1995*a, b*) and molecular genetic research investigating regional differences in activity of the *FMR1* gene in the developing and mature mammalian brain (Abitbol et al. 1993; Hergersberg et al. 1995; Hinds et al. 1993). Specific brain regions, including the superior temporal gyrus and hippocampus (Reiss et al. 1994) also show age-related volume changes in individuals with fragile X that are not observed in controls (Reiss, Abrams, et al. 1995; Reiss, Freund, et al. 1995; Reiss et al. 1994).

Localization of brain regions where absence of the *FMR1* protein appears to have the greatest effect is suggested by findings from both imaging and molecular genetic studies. Although we can offer hypotheses implicating dysfunction of these regions in the pathogenesis of neurobehavioral abnormalities in individuals with fragile X, it is of great importance that the link between neuroanatomy and neurobehavior be confirmed with studies of brain function.

However, to date, only one functional brain imaging study of 10 individuals with fragile X syndrome has been performed (Schapiro et al. 1995). This study used positron emission tomography (PET) to measure resting regional cerebral metabolic rates for subjects with fragile X and comparison groups consisting of normal controls and individuals with Down syndrome. Relative to the two comparison groups, the fragile X group had greater metabolic activity in the right parietal lobe, right calcarine region, and right caudate nucleus. Activity of the cerebellar vermis also was significantly elevated in the fragile X group.

Overall, Schapiro's study is limited by the small number of subjects and the absence of any stimuli or tasks for regional activation. We have initiated functional brain imaging studies that will use more subjects, specific activation tasks to elucidate normal and abnormal brain function in affected individuals, a unique comparison group consisting of individuals with the *FMR1* premutation (as well as normal controls), and acquisition and analysis of data obtained from the same activation paradigm and subjects, using two different functional imaging modalities (fMRI, PET).

It is essential that additional information be obtained about the mechanisms by which the *FMR1* mutation affects brain development and function. These data are needed to develop a better understanding of the neurobiological basis of neurobehavioral dysfunction in affected individuals, the neurodevelopmental sequences during which the *FMR1* protein is most critical, the development and testing of more effective pharmacological, and, eventually, genetic interventions for children and adults with this condition.

Genetic and Environmental Influences

The independent and interactive effects of genetic and environmental influences on human cognition and behavior are complex and include issues related to shared and nonshared environment, genotype-environment interaction, nonadditive genetic variance, and changes in the magnitude of genetic influence in relation to child development, cognitive ability, and neuropsychological domain (Plomin and Neiderhiser 1991). Advances in molecular genetics have accelerated the pace of quantitative genetics research, with particular emphasis on the identification of specific genes that contribute to variation in normal human cognition and behavior (Plomin et al. 1994).

Although not without controversy, research aimed at the evaluation of genetic and environmental influences in children with normal IQs has gained in importance over the past two decades. Similarly, in the case of fragile X syndrome, where a known genetic abnormality adversely affects cognition and behavior, the identification of those domains of function that are most influenced by environmental or genetic factors is of primary importance. Knowledge of the neurobehavioral and neurobiological domains where the *FMR1* mutation has its greatest impact will confirm the specificity of characteristics comprising the fragile X phenotype versus attributes associated with nonspecific, general developmental disability.

Knowledge of the domains of neurobehavioral functioning that are most influenced by the *FMR1* mutation has direct relevance for identifying dysfunctional modes of learning or behavior that may be best addressed with alternative methods of information processing or analysis. Neurobehavioral domains that are highly influenced by genetic factors could also be prioritized for monitoring the effects of biological interventions such as specific pharmacological agents (e.g., antianxiety medications, cognitive enhancing agents) and specific genetic therapies which might be developed in the future.

Equally important is the knowledge of those neurobehavioral domains that are most directly influenced by characteristics of the home or school environment in children with the fragile X syndrome. This information can be used to adjust the home and school settings so as to optimize the neurobehavioral outcome of the affected child. For example, if educational opportunity has a major impact on neurobehavioral outcome, then investment in early and sustained special educational intervention may significantly decrease future morbidity in children with fragile X syndrome. Similarly, identification of factors related to family function that can adversely influence child behavior will help to target those areas that, if addressed effectively through therapeutic intervention, may have maximal impact on improving the function of children affected with fragile X and their families.

Gene, Environment, and Neurobehavioral Function

In an initial study designed to investigate the association of *FMR1* gene activation and parental IQ with neurobehavioral outcome (Abrams et al. 1994), young females with the fragile X full mutation were assessed using quantitative measures of mutation amplification size (Amp) and the ratio of active normal X chromosome to total normal X chromosome (activation ratio—ActR). To investigate molecular-behavioral associations, Amp and ActR were used as independent variables, while IQ scores were used as dependent variables.

Significant correlations were observed between both molecular variables and measures of cognitive functioning, with ActR showing the most consistent and robust correlations. As ActR increased, overall IQ and specific subtest and area scores from the cognitive tests increased. In a followup study (Reiss, Abrams, et al. 1995; Reiss, Freund, et al. 1995), the proportion of variance predicted by average (mid) parental IQ (MPIQ) decreased across all cognitive measures in a group of girls with the fragile X full mutation compared to a matched nonfragile X group. The contribution of ActR to intellectual function in girls with the *FMR1* full mutation was best modeled as a graded effect, particularly for those cognitive measures that distinguish fragile X from nonfragile X groups (e.g., visual-spatial, attention). This is in contrast to AMP, which showed little or no correlation with cognitive outcome. These data suggest that activation of the *FMR1* gene is directly linked to the severity of intellectual dysfunction in girls with the *FMR1* full mutation, particularly for the visual-perceptual and attentional- organizational domains.

Preliminary analyses also were carried out to investigate the relation of the home environment and socioeconomic status (SES) to cognitive outcome in 30 girls with fragile X and 30 controls matched on gender, age, IQ, and SES. Hierarchical/stepwise regression analyses were used to evaluate the independent contributions of specified predictor variables to cognitive outcome in both groups. Independent variables used in these analyses included MPIQ, SES (Hollingshead 4-factor), ActR, and three subscale scores from the Family Environment Scale (Moos and Moos 1994).

The analyses suggest that the profile and magnitude of environmental influences on cognitive outcome may be different in families having a child with fragile X syndrome than in nonfragile X families. SES factors considered to be consistent with a home environment enhanced for academic success were significantly correlated with cognitive scores in the comparison group but not in the fragile X group.

In comparison, SES predicted a small, but significant proportion of the variance in verbal scores in the fragile X group. As verbal skills in children are most influenced by educational factors, this finding may, in part, be related to better educational opportunities afforded by higher SES (e.g., availability and adequacy of special education). The correlation between SES and verbal scores in children with fragile X also may be related to the finding that deleterious effects of the full mutation may be relatively less for this cognitive domain compared to domains represented by the other two factors or summary scores (Reiss, Abrams, et al. 1995; Reiss, Freund, et al. 1995).

Although the data presented here are preliminary, they indicate that a more comprehensive analysis of the association of genetic and environmental factors with neurobehavioral outcome is warranted in children with this common genetic condition. To accomplish this task, we plan to evaluate a larger number of subjects with a study design that will permit linear structural equation modeling. The information derived from this study could be of direct benefit to the design of more specific biological and environmental mediation strategies.

Gene, Brain, and Behavior

In a recent study (Reiss, Abrams, et al. 1995; Reiss, Freund, et al. 1995), we showed that caudate and lateral ventricular volumes were increased in individuals with the fragile X full mutation when compared to controls matched on age, gender, and IQ. We analyzed the correlation of these regions with measures of *FMR1* function and neurobehavioral outcome as a means of testing the validity of the findings of selective abnormalities in brain morphology in individuals with fragile X syndrome. These analyses showed that both caudate volume and ventricular volume were correlated with the methylation status of the *FMR1* gene in the expected direction, that is, larger (more aberrant) volume was associated with decreased activation of the unaffected X chromosome (i.e., decreased ActR). Caudate and lateral ventricular volumes also were negatively correlated with IQ.

In a separate report on brain-behavior and gene-behavior links, we showed that the size of the posterior-inferior cerebellar vermis in girls with the *FMR1* full mutation was significantly (negatively) correlated with the severity of autistic behaviors, particularly for those DSM-III-R criteria relating to aberrant language and stereotypic behavior/unusual responses to sensory stimuli (Mazzocco et al. in press). Analysis of the association between ActR and severity of autistic responses to sensory stimuli behavior also revealed that decreasing activation of the normal *FMR1* gene was significantly associated with greater severity of autistic behavior.

Conclusion

Our initial attempts to describe gene-behavior, gene-brain, and brain-behavior associations in individuals with the fragile X full mutation indicate clearly that such studies are feasible and productive. Our preliminary results suggest that statistically significant and biologically intriguing correlations exist among a putative measure of *FMR1* gene activation (ActR), the morphology of particular brain regions, and measures of cognitive function or behavior.

To date, most of the genetic data apply to the study of fragile X females, in whom the activation status of the *FMR1* gene depends largely on the process of normal X chromosome inactivation (i.e., Lyonization). Although Lyonization does not occur in the male with fragile X, genotypic differences in the form of variation in CGG repeat length and proportion of methylated alleles (i.e., mosaicism) does provide a potential basis for phenotypic variation.

Results from ongoing studies in our Research Center will help to fill an important gap in our knowledge concerning the effects of variation in the *FMR1* mutation in young males. Although repeat length and methylation status have been useful for analyses carried out to date, more direct measures of *FMR1* gene function also are needed. This is particularly important as there is increasing evidence that many, if not most, males with the fragile X syndrome are genetic mosaics, carrying both premutation and full mutation alleles (Oostra and Verkerk 1992). Accordingly, we are currently measuring *FMR1* protein levels in our fragile X subjects in addition to our current molecular variables.

With respect to cognitive function, the organization of the fragile X brain appears to differ from normal children. However, due to the lack of data on the youngest identifiable children with the *FMR1* mutation, the timing of the onset of this difference is currently unknown. Ongoing studies in our Research Center will help to clarify when the fragile X brain becomes structurally and functionally different from the controls.

The study of homogeneous disorders such as the fragile X syndrome provide a powerful method of scientific inquiry into human gene-brain-behavior linkages that complements more traditional research approaches. In particular, the use of these disorders as models of common behavioral and cognitive conditions occurring in the general population can reveal insights into neurodevelopmental pathways that might otherwise be obscured or diluted when investigating more heterogeneous subject groups.

A basic premise of behavioral neurogenetics research is the need for investigation at multiple scientific levels of inquiry, including quantitative assessment of genetic factors, brain structure and function, and neurobehavioral processes. Such research requires in-house expertise for each of these levels of inquiry or productive collaborations with colleagues interested in similar approaches to scientific investigation. In addition to fragile X, research ongoing in our center includes the study of individuals with Turner syndrome, neurofibromatosis, Tourette syndrome, Down syndrome, sex chromosome aneuploidy, and other genetic conditions.

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Commentary

Synapse Stabilization and Fragile X Protein Synthesis in the Rodent Brain

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The presentations by Rakic and Reiss have provided an excellent basis for my role as a discussant of this session. Rakic spoke of the now well-known phenomenon of synapse overproduction and selection in sensory system development. It has been established in the cat, monkey, and human visual systems that an excess of synapses is generated relatively early in postnatal development and that the selective preservation of functionally "appropriate" synapses and the elimination of inappropriate synapses is a mechanism whereby pattern is established in the synaptic network (e.g., Boothe et al. 1979; Cragg 1975; LeVay et al. 1980).

This pattern of synapse overproduction and loss is quite general throughout the developing primate brain, as Rakic noted, and there is debate as to whether the loss process is more or less synchronous throughout the brain, as Rakic argued, or whether it varies with the developmental schedule of individual brain regions, as Huttenlocher and his collaborators have proposed (e.g., Huttenlocher 1994).

It should be noted that earlier comparisons of human and monkey data were based on synaptic density measures, variations in which may not reflect solely changes in synapse numbers. Subsequent papers have incorporated various corrections, such that the data more closely reflect actual synapse numbers (e.g., Bourgeois and Rakic 1993; Huttenlocher 1990, 1994), and the results continue to show different temporal maturation patterns for humans and synchronous maturation for the rhesus macaque.

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It is important to consider the functional purpose of this synapse overproduction and the subsequent experience-based selective stabilization of a subset of synapses. Evidence indicates that it is an information capture mechanism designed to organize the developing brain on the basis of predictable regularities in the sensory environment of the neonate. Black and Greenough (1986) termed such mechanisms "*experience-expectant*," reflecting the brain's readiness to receive information that has been reliably available throughout the period during which the developmental process evolved.

The classical evidence for this is the work indicating that the development of normal binocular organization of the visual system requires balanced input via the two eyes, such that overlapping visual cortical afferents are eliminated and columnar regions of dominance for each eye are preserved (LeVay et al. 1980). The LeVay et al. work also showed that "plasticity cuts both ways" (as J. McV. Hunt often argued) and that the developing system captures whatever information is available, resulting in maldevelopment in a distorted sensory world. The work described by Rakic and by Huttenlocher (1994) indicates that patterns of overproduction and loss of synapses are widespread in the primate brain, including humans, and suggests that experience-expectant development may be the rule, not the exception, in the early development of basic emotional and cognitive as well as sensory and motor systems.

In comparison to primates and cats, there is considerably less evidence for overproduction and loss in the rodent brain. Developmental studies have indicated little or no loss of synapses in rat cerebral cortex, but rather a gradual accretion of synapses to an asymptotic level (Aghajanian and Bloom 1967; Blue and Parnavelas 1983). These studies did not use appropriate stereological correction, and hence their quantitative conclusions could have been affected by other changes, such as in synapse size or in the volume of other components in the case of the Aghajanian and Bloom study.

Quantitative studies of spine density in Golgi-impregnated tissue have reported some loss of spines in rat cortex across late development (e.g., Miller 1981; Juraska 1982), and there is evidence for late axonal withdrawal in the corpus callosum (Kim and Juraska 1994). Nothing reported in the rat, however, is as dramatic as that reported for primates (e.g., Boothe et al. 1979; O'Kusky and Colonnier 1982; Bourgeois and Rakic 1993; Huttenlocher 1990, 1994). It is possible that this reflects a fundamental difference between the developmental processes of the rodent and the primate brain, or as Huttenlocher (1994) proposed, a phylogenetic trend toward increased synapse loss across rodents, felines, and primates. Alternatively, it is possible, as suggested by the asynchrony of axon and spine loss across different cell types within the rat visual cortex (Juraska 1982; Kim and Juraska 1994), that the time compression of rodent brain development results in overlapping synaptogenesis and synapse elimination processes, such that the elimination is masked by the continuing formation of synapses.

Greenough and Chang (1988) addressed this issue, taking advantage of the

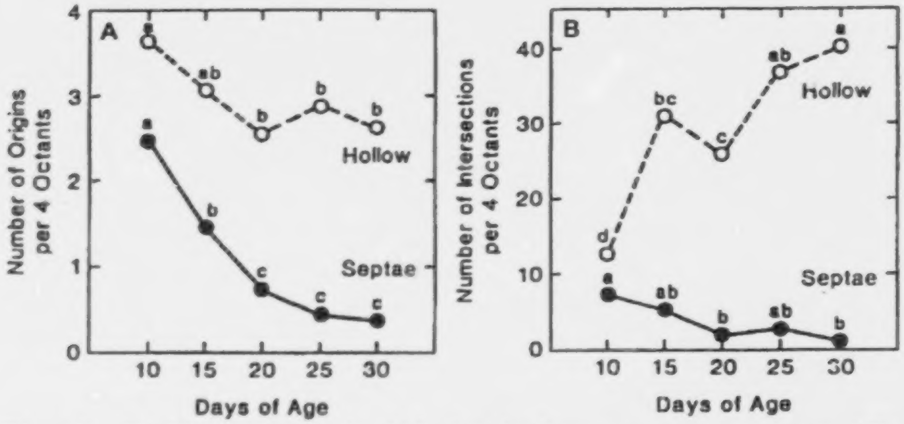

Figure 1 Dendrite development in whisker barrels of mouse somatosensory cortex. *A* The number of dendrites originating from barrel wall neurons on the side oriented toward the hollow versus the side away from the hollow. Dendrites oriented away from the hollow are much more likely to be eliminated as the mouse matures. *B* "Intersections," reflecting dendritic mass, increase in the region toward the hollow while decreasing in the region away from the hollow. Without the orientation information provided by the barrel configuration, the cells would be seen as gradually adding dendrite (from Greenough and Chang 1988).

regularities of the cellular organization of the posteromedial barrel subfield of the mouse somatosensory cortex. In this region, neuronal somata in layer IV are arranged in rings termed "barrels" (Woolsey and Van der Loos 1970) that surround the bulk of their dendritic fields, which interact with thalamocortical axons in the hollow of the barrel. In the adult, spiny stellate neurons project the bulk of their dendrites in the direction of the barrel hollow, and only a small amount of dendrite typically extends in other directions, particularly in the opposite direction toward the septae between barrels.

Greenough and Chang (1988) examined the development of this oriented pattern in Golgi-impregnated tissue, quantifying the amount of dendrite oriented toward the barrel hollow versus that oriented toward the interbarrel septae at ages ranging from 10 to 30 postnatal days. Figure 1*A* depicts the relative number of branches with these orientations originating from the soma, and figure 1*B* depicts the amount of dendrite (determined using a ring intersection analysis) at the two orientations. It is clear that branches originating in both orientations are lost during early development, but far more of those emanating toward the septae are lost. We were unable to define barrels sufficiently well to examine dendrite orientation in a sample of mice from day 6 PN, but one can imagine from figure 1*A* that the pattern of branching at that time might be nearly symmetrical.

Figure 1*B* clearly indicates that in measures of the overall growth of the dendritic field, this loss would be masked without the information provided by the dendrite's orientation in relation to the barrel hollow. The "dendritic mass" extending away from the hollow decreases progressively with age at the same time that the amount of dendrite extending toward the barrel hollow is increasing, and in the absence of the positional information, the average overall dendritic growth for these cells would be a pattern of consistent accumulation of dendrite. The fact that dendritic regression can be masked by dendritic growth in the mouse somatosensory cortex suggests that other facets of synapse production and loss might similarly be masked in studies of this and other rodent neocortical areas.

A further and somewhat more speculative suggestion, which is certainly compatible with our knowledge of the development of early axonal projections (e g., Stanfield et al. 1982), is that synapse overproduction and subsequent selection is a very widespread process in the nervous system, with the numbers of "tentative" connections being vastly greater than might be supposed from the quantification of connections at any point in time. Taken with other work indicating possible overproduction in nonneocortical systems (e.g., Weiss and Pysh 1978), it appears that experience-expectant processes may be an even more prominent component of brain development than we have heretofore believed.

A second component of the development process, which extends across the lifespan, is termed *experience-dependent information storage* (Black and Greenough 1986). In this process, experience appears to drive the formation of synapses. Two models for this are adult exposure to a complex environment (e.g., Juraska et al. 1980) and adult motor skill learning (e.g., Black et al. 1990), in which synapses appear to be added more or less proportionately to the amount of learning that takes place (Kleim et al. 1996). In contrast to experience-expectant development, there is no evidence for synapse overproduction and loss in the adult case, although there is little evidence against it as well.

Dr. Reiss focused much of his presentation on a seemingly unrelated phenomenon, the fragile X mental retardation syndrome. The fragile X syndrome, as Reiss noted, is caused by an excessively long repeat of the triplet codon CGG in a region 5′ of the first exon of the FMR1 gene, which is on the X chromosome. The repeat both inhibits transcription and impedes translation of the gene leading to reduced or absent expression of the fragile X mental retardation protein (FMRP) and associated mental retardation (Warren and Nelson 1994; Feng et al. 1995). Less widely known is that the syndrome is also associated with deficits in social behavior (e.g., gaze avoidance) and often exhibits comorbidity with autistic behavior, schizotypal symptoms, and depression (Baumgardner et al. 1995; Franke et al. 1995; Kerby and Dawson 1994; Sobesky et al. 1994).

Our contact with this syndrome occurred by chance. Following up on reports that polyribosomal aggregates (PRAs), the cellular sites of protein synthesis, were more frequently found in spines during reactive synaptogenesis (Steward and Levy 1982; Steward and Falk 1986), we found that synapses in visual cortex of developing rats exhibited PRAs in spines much more frequently than in mature rats, suggesting an association with some aspect of the synaptogenesis or synapse maturation process (Hwang and Greenough 1984). Subsequently, we found that synapses in the visual cortex of rats housed in a complex, toy-filled group environment, where considerable synaptogenesis takes place, were more likely to have PRAs in spines (Greenough et al. 1985). This led us to develop a synaptoneurosome preparation from P 12–16 rat cerebral cortex, adapting the method of Hollingsworth and associates (1985).

Using this system, we were able to show that K+ depolarization or glutamate addition caused a rapid increase in the amount of RNA in the polyribosome fraction and increased synthesis of protein (Weiler and Greenough 1991). Subsequent work showed that the glutamate receptor involved is a metabotropic receptor of the subgroup that is coupled to phosphoinositide hydrolysis (mGluR1 and mGluR5). mGluR stimulation activates an inositol phosphate-protein kinase C cascade that leads, through still undetermined mechanisms, to polyribosomal aggregation and protein synthesis (Weiler and Greenough 1993). Since specific PKC blockers impede this reaction, it appears that protein synthesis at synapses can be regulated by synaptic activity via the phosphorylation state of some as yet unidentified proteins. The conditions that regulate this synthesis in vivo remain to be determined.

It is of obvious interest to know which protein(s) are synthesized in this manner at synapses. We have begun this process in collaboration with Jim Eberwine's laboratory (Miyashiro et al. 1994), which has prepared cDNA libraries to mRNA fragments from single neurites of cultured hippocampal neurons. We used members of this library as probes to determine whether any of the corresponding mRNAs are taken up into newly assembled polyribosomes, using sucrose density gradient sedimentation to separate PRAs by size following glutamate receptor agonist stimulation of synaptoneurosomes. We have so far found two messages that appear to associate rapidly with ribosomes in response to metabotropic receptor activation. One of these appears to have homology to a recently identified autosomal homolog of FMR1 called FXR1 (Siomi et al. 1995). Following up on this lead, we used three synthetic oligonucleotide probes designed from the sequence of FMR1 to probe sucrose gradient fractions; we find that mRNA for FMR1 appears to be translated at synapses in response to mGluR agonist stimulation, as well (Weiler et al. 1997).

Because of this association with ribosomes, we used a direct test for the translation of the FMR protein (FMRP) in synaptoneurosomes. We used the mAbla antibody of Devys et al. (1993; from Chemicon) to probe Western blots from synaptoneurosome preparations that were stimulated with the specific mGluR agonist DHPG for 2 or 5 minutes, compared to unstimulated controls. Bands corresponding to four isoforms of FMRP were all more intensely immunoreactive in the stimulated groups. Immunostaining for glial fibrillary acidic protein as a control contaminant present at low levels in the preparation and unlikely to respond to receptor activation indicated no effects of DHPG stimulation (Weiler et al. 1997). This indicates that FMRP is synthesized at synapses in response to neurotransmitter activation of metabotropic glutamate receptors.

The function(s) of the FMR–FXR gene family remain unknown. Members of the family dimerize and contain domains that bind relatively promiscuously with mRNA (Zhang et al. 1995); in addition, the fragile X protein has been shown to bind to ribosomes (Khandjian et al. 1996). A plausible function is involvement in some aspect of the regulation of protein synthesis.

Another piece of evidence regarding function, which helps to tie the work presented by Dr. Rakic to that presented by Dr. Reiss, arises from morphological studies of synapse structure in fragile X autopsy cases and from our confirmatory studies of the fragile X knockout mouse produced by Oostra and Willems (Consortium 1994). The gross morphology of the brain in fragile X cases exhibits subtle differences as Reiss has noted, with some structures being larger in the brains of fragile X patients. At a microstructural level, the fragile X neocortex is characterized by long, thin, sometimes tortuous or twisting dendritic spines (Rudelli et al. 1985). These spines appear similar to those present in relatively early development, as if they were unable to take on the normal, mature form.

We have similarly quantitatively evaluated spine length and spine density along the apical dendrites of layer V pyramidal neurons from visual cortex of these fragile X knockout mice. As figure 2*A* indicates, spines were longer, on average, across most of the range of the spine length distribution in the knockout mice, paralleling the elongation seen in human clinical autopsy cases. Perhaps even more functionally revealing, however, is the spine density distribution depicted in figure 2*B*, which shows the density of spines to be higher in the fragile X knockout mice than in the wild-type mice.

This result suggests that the synapse elimination process may be impaired in the fragile X knockout mice, a finding compatible with the hypothesis that fragile X protein synthesis at synapses is involved in synapse stabilization, although not necessarily supportive of it. If synapse stabilization and elimination were somehow coupled, as they appear to be in the competitive synapse elimination process in the primate and feline visual systems, then an impairment in a synapse maturation-stabilization mechanism could presumably affect the synapse elimination process as well. This finding is also potentially compatible with Reiss's statement that some brain regions are larger in fragile X patients.

The manner in which FMRP carries out whatever actions it performs at the synapse is likely to involve interactions with RNA and probably the protein synthesis process. FMRP (and its autosomal homologs) contain two KH domains that bind messenger RNA (Siomi et al. 1994). Another region of the protein binds to ribosomes (Khandjian et al. 1996). A function such as regulation of the synthesis of other proteins seems likely.

At this point, the absence of data leaves ample room for speculation. Certainly synapse maturation and elimination mechanisms could be involved in the cascade-like spread of the effects of multifocal cortical abnormalities to

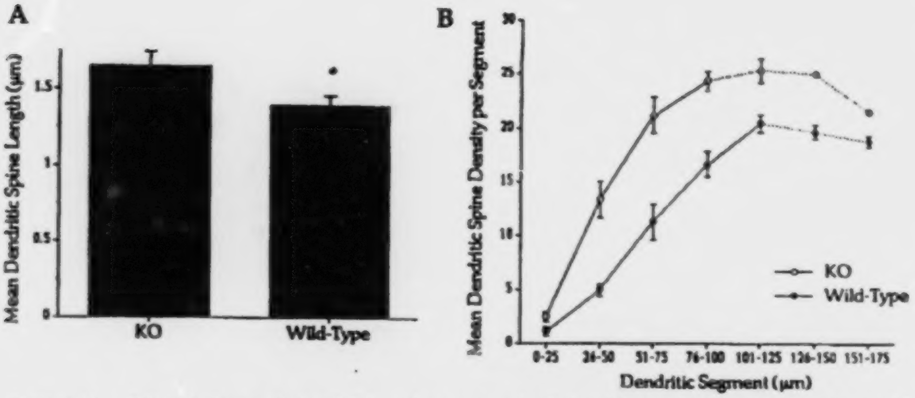

Figure 2 Morphology of dendritic spines on apical dendrites of visual cortex layer V pyramidal neurons in fragile X knockout and normal mice produced by Oostra and Willems (Consortium 1994). *A* Spine length is greater for the knockout mice, paralleling the human syndrome (Rudelli et al. 1985). *B* Spine density is greater, suggesting possible impairment of a synapse elimination process (data from Comery et al. 1997).

afferent and efferent regions as described by Dr. Galaburda. We and others (Black and Greenough 1986) have noted the parallel between the apparent loss of phonemic boundaries that appears to be driven by experience during development (e.g., Werker and Polka 1993) and the process of synapse elimination/selection. This clear evidence for a role of experience in the development of components of language is compatible with Dr. Bates' emphasis on the role of experience in language development.

A final point relates to the findings that deficits in social behavior and symptoms characteristic of schizophrenia and depression are frequently exhibited by fragile X patients. It is well known that rhesus monkeys deprived of normal social contact during development exhibit deficiencies in social behavior and other behaviors (e.g., stereotypies) that parallel some symptoms of affective disorders. It seems at least plausible that a deficiency in synapse stabilization associated with the encoding of early social experiences could contribute to aberrant psychosocial behavior as well as to mental retardation.

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Social Behavior

Toxic Environments, Neuronal Plasticity, and Delinquency

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ABSTRACT

One of the most disturbing aspects of our society is the concentration of individuals with academic underachievement, unemployment, substance abuse, and high risk for violence in areas of urban impoverishment. That this pattern of maladaptive behavior may persist over generations has led some to conclude that genetic factors play a significant role in its etiology. However, the influence of "toxic" environments on brain and social development are not adequately addressed. First, the high prevalence of fetal exposure to substances of abuse–ethanol and drugs–adversely affects brain development. Second, a disproportionate risk for exposure to lead can disrupt in a relatively irreversible fashion cognitive functions and attention in a way that promotes impulsive and aggressive behavior. Third, repeated exposure to traumatic experiences can cause hippocampal atrophy and secondary neurocognitive impairments. The molecular mechanisms responsible for these three forms of developmental brain insults are increasingly better understood. This research has important implications for treatment, prevention, and policy so that this cycle of failure can be broken.

In considering the response to the invitation to discuss neuronal plasticity and social behavior, I decided to draw upon my clinical experience in child psychiatry and my research background in neurotoxicology and brain development to address an issue of considerable societal significance—the nongenetic

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causes of cognitive impairments and delinquency. This issue stands at the very epicenter of the debate over the relative salience of genetic determinism versus environmental explanations of psychopathology.

Substantial progress has been made by the Human Genome Project in mapping and sequencing of those one hundred thousand genes on the human chromosomes, 60 percent of which are related to the nervous system. In the last few years, the genetic causes of several disorders that affect cognition, including Fragile X, Huntington's disease, and Alzheimer's disease, have largely been resolved. Recently, heritable variants of the gene encoding for a subtype of receptor that mediates the action of the neurotransmitter dopamine, the D-4 receptor, has been associated with extroverted personality and with attention deficit hyperactivity disorder (Benjamin et al. 1996). The recent progress in neural and behavioral genetics is impressive and is accelerating.

But my enthusiasm for reductionistic explanations for complex behaviors, including developmental problems of cognition, is tempered by my 15 years in child psychiatry. In my experience in Baltimore and more recently in Boston, hospital units dedicated to the treatment of children with severe mental disorders contain a disproportionate number of children from the inner cities with learning disorders, severe problems of impulse control, and delinquent behavior known as conduct disorder. The most serious, recalcitrant, and personally disturbing disorders are not schizophrenia or depression in childhood but the residue of inner city life.

The litany of the statistics is chilling. In our inner cities, AIDS, homicide, and suicide are the leading causes of death for male youth. Single mothers, often with the first birth in her early adolescence, are the norm. In the District of Columbia, over one-third of young African-American males are in the criminal justice system. Surveys indicate that nearly half of the children in certain inner city neighborhoods have witnessed or personally known an individual who was murdered. Our societal response to these problems that plague our inner cities is clear—increase the prison system. The budget for the penal system in California, for example, will soon exceed that of its university system.

It would be hoped that government policy would be shaped by secure knowledge—that is, empirical evidence. Analysts clamor for attention to shape policy. For example, Hernstein and Murray (1994) in "The Bell Curve" offer an analysis that has garnered considerable public attention. They state: "For most of the worst social problems of our time, the people who have the problem are heavily concentrated in the lower portion of the cognitive ability distribution."

The second element of their argument rests on the heritable determinants of intelligence: "Cognitive ability is substantially heritable, apparently no less than 40 percent and no more than 80 percent." They argue that heritability of intelligence accounts for the lower intelligence and consequent maladaptive social behavior concentrated in the inner cities, especially among minorities. While paying lip service to environmental factors, their conclusion is that genetics is destiny. This conclusion is seemingly reinforced by the observation that underachievement appears to persist from one generation to the next in the inner city. Obviously, this conclusion has major policy implications.

Even accepting their assessment of IQ, their conclusions violate the very statistical basis of their argument—IQ in the population does not exhibit a normal distribution or bell-shaped curve. Rather, it is skewed on the low end, with a disproportionate representation of individuals with subnormal intelligence as assessed by standard testing methods. Aside from reservations about cultural and language biases inherent in IQ assessment, the skew in lower levels of IQ indicates that inferences about heritability of intelligence developed from studies carried out principally in individuals within the normal range of intelligence have little applicability to this population. Keeping in mind the seductive use of "on average" by Hernstein and Murray to designate a low-IQ population that is composed of a very heterogeneous group of individuals, I would suggest that "toxic environments" play a significant role in limiting optimal development and fostering social dysfunction and that these adverse circumstances create self-perpetuating, transgenerational dysfunction.

The particular problem on which I am focusing concerns delinquent behavior or, as it is clinically designated, conduct disorder. The diagnostic criteria for conduct disorder established in the current "Diagnostic Statistical Manual IV" are based upon epidemiologic studies to ensure a high level of validity. Importantly, conduct disorder is one of the most reliable diagnoses in child psychiatry and has a high level of predictability with regard to adverse outcomes (Robins 1991). In addition, a number of other clinical conditions often cooccur with conduct disorder, including attention deficit disorder with hyperactivity, depression, and substance abuse.

The risk factors for conduct disorder are diverse but are well characterized. Notably, genetic factors appear to make a minimal contribution, whereas cognitive impairment is an important variable, along with social circumstances (Mrazek and Haggerty 1994). The nongenetic factors that increase the risk for cognitive impairment in children include adolescent pregnancy, poor perinatal care, perinatal substance abuse, toxin exposure, and neglect.

Recent advances in developmental neurobiology and neurotoxicology are shedding considerable light on the underlying mechanisms associated with these risk factors that interfere with brain development and can account for developmental cognitive impairments and behavioral disorders. The elucidation of these mechanisms is important, because understanding them should have significant implications with regard to the formulation of national policy and the development of preventive strategies.

One of the fundamental discoveries of neuroscience is that neurons communicate with each other by means of chemical messengers (Hyman and Nestler 1993). The chemical messenger is released by the nerve and activates receptors on the adjacent neuron, thereby transferring information. The neurotransmitter receptors may be linked to ion channels through which sodium, calcium, or chloride ions flow, or they may be linked to the generation of intracellular second messengers that produce a more persistent alteration in neuronal activity. An important recent advance in our understanding of chemical neurotransmission is that activation of neurotransmitter receptors can ultimately affect gene expression in neurons, so that information flow in the nervous system can exert significant and persistent changes in brain gene expression. The toxins of interest to this discussion alter chemical neurotransmission in brain.

One toxin that has had considerable sway in our inner cities is cocaine. In one study, up to 30 percent of inner city residents who volunteered for a urinary screen tested positive for cocaine metabolites (Dempsey et al. 1996), whereas cocaine use has exhibited a remarkable decline in the general population. The problems associated with fetal brain exposure to cocaine are complex. First, cocaine is rarely used alone but is often used in combination with alcohol, nicotine, and opiates so that determining the specificity of adverse effects is difficult. Second, it is difficult to track cocaine abuse in pregnancy because the use may not be continuous. Third, cocaine has a variety of noxious effects, including affecting placental blood flow, aside from interfering with brain chemical neurotransmission. Finally, cocaine abuse in women is often associated with poor perinatal care and malnutrition.

The mechanism of action of cocaine in brain is very well understood at the cellular and molecular level. It blocks the inactivation of the neurotransmitter dopamine by binding to the presynaptic dopamine transporter which removes dopamine from the synapse. The enhanced action of dopamine accounts for cocaine's euphoriant action and high liability for addiction (Hyman and Nestler 1993). The neurotransmitter systems with which cocaine interacts—dopamine and to a lesser extent serotonin and norepinephrine—are formed quite early in brain development. Studies in the fetal rat brain demonstrate that these neurotransmitter systems send processes to innervate forebrain structures at the earliest stages of cortical development—by 15 days gestation (Coyle 1977). This developmental stage in the rat is roughly equivalent to the end of the first trimester in human fetal development.

Developmental neurobiology is now showing that the very neurotransmitters involved in information processing in the adult brain play a critical role in regulating neuronal maturation in the developing brain. The potential role of the dopamine receptors in modulating these processes has been elucidated in cell culture studies of rat frontal cortex (Todd 1992). Activation of dopamine receptors on fetal cortical neurons significantly enhanced neuritic extension and process branching. At first blush, enhancement of neuronal maturation may seem like a positive effect of cocaine's overstimulation of dopamine receptors. However, in the tightly choreographed development of the brain, timing is everything. Thus, premature differentiation may foreclose precisely timed interconnections that occur later in development. Indeed, in a recent study, Reinoso et al. (1996) showed that the structural abnormalities of neurons located in dopamine-rich areas of the brain persist into adulthood after fetal cocaine exposure.

Prolonged overstimulation of a receptor in the nervous system generally results in a compensatory desensitization of the receptor. When such desensitization occurs early in development, it may result in much more persistent downregulation of receptor responsiveness. For example, in a very well characterized and controlled mouse model for fetal cocaine exposure, Kosofsky et al. (1996) found that dopamine receptors in the cerebral cortex and in the striatum are significantly hyporesponsive after birth in fetuses exposed to cocaine in utero. Hyporesponsiveness of one type of dopamine receptor, the D-4 receptor, has been implicated in novelty-seeking behavior (Benjamin et al. 1996). Indeed, Kosofsky and his colleagues (1996) demonstrated a number of structural and behavioral abnormalities specific to fetal cocaine exposure in controlled studies of mice in a learning paradigm. The mice exposed to cocaine in utero exhibit an impulsivity that correlates with persistent abnormality in dopaminergic neurotransmission and is consistent with behavioral problems associated with fetal cocaine exposure in humans.

Another developmental toxin, the significance of which has only recently been appreciated, is alcohol. Contrary to the bell curve, the risk of fetal exposure to toxic levels of alcohol is not random nor uniformly distributed in our culture. Fetal alcohol syndrome is typically diagnosed by the presence of dysmorphic features including wide set eyes, small central face, low birthweight, and cognitive impairment. A recent study by the Institute of Medicine indicates that the prevalence is highly variable and may be underreported (Stratton et al. 1996). For example, reports to birth defect registries suggest a prevalence of 0.2 per 1,000 births in the United States, whereas studies of all births in clinics suggest a rate of 1 to 3 per 1,000. In contrast, a population-based study of a Native American tribe with a high rate of alcohol abuse revealed 120 cases per 1,000.

The molecular sites of action of alcohol in the nervous system have recently been clarified (Tsai et al. 1995). Aside from enhancing the action of the inhibitory neurotransmitter, GABA, ethanol affects the major excitatory neurotransmitter in brain at a subclass of its receptors, the NMDA receptor. Ethanol noncompetitively inhibits the response of the NMDA type of glutamate receptor in a dose-dependent fashion, with rather striking inhibition occurring at concentrations associated with inebriation. The NMDA receptor is at the epicenter of excitatory neurotransmission in brain and is responsible for cognitive processing, the laying down of memories, and the modification of neuronal connectivity as a consequence of experience. But during development, the NMDA receptor subserves additional roles. Activation of the NMDA receptor stimulates neuronal migration in the developing nervous system and has trophic effects on immature neurons so that they can differentiate and mature. Excessive activation of the NMDA receptor leads to neuronal degeneration.

Recent studies have taken advantage of our emerging understanding of the role of the NMDA receptor in the developing nervous system to identify mechanisms of ethanol toxicity, aside from nutritional deprivation, that would selectively interfere with brain development. These mechanisms include blockade of the neurotrophic effects of the NMDA receptor, which could lead to neuronal death in the fetal brain, and blockade of long-term potentiation, whereby NMDA receptors modify synaptic efficacy. In addition, as a consequence of upregulation of NMDA receptors due to persistent inhibition by ethanol, neurons with overreactive NMDA receptors may degenerate as a consequence of the abrupt ethanol withdrawal that invariably occurs at birth.

A third toxin that unequivocally impairs cognitive development is lead. The risk for lead toxicity is not uniformly distributed in our society but rather affects certain groups much more than others. A recent study examined blood load levels and their sociodemographic determinants in the US population (Brody et al. 1994). Non-Hispanic blacks from ages 1 to 11 had lead levels on average that were twice that of non-Hispanic whites.

Needleman et al. (1996) published a study in which they exploited an x-ray technique to measure bone lead. The power of this approach is that it provides a cumulative record of lead exposure as opposed to blood lead levels, which may fluctuate with changes in living circumstances or diet. They stratified children on the basis of bone lead levels in the analysis of behavioral data. Those with higher lead levels were twice as likely to have clinically significant problems with aggression, delinquency, and attention, whether rated by parents or by teachers, than those with lower lead levels. Obviously, many factors can contribute to these behavioral problems in inner city youth, but a doubling of their prevalence as a correlate of cumulative lead exposure points to a major risk factor.

The mechanism of central nervous system (CNS) toxicity of lead is complex, since it interacts with a wide variety of processes that are ordinarily activated by calcium. Markovac and Goldstein (1988) have shown that lead was several orders of magnitude more potent at activating the enzyme protein Kinase C than the endogenous activator calcium. Protein Kinase C plays a central role in signal processing in the nervous system, and its persistent activation, especially during brain development, would have deleterious effects on brain maturation (Kaczmarek 1987).

These three examples represent only a few of the noxious effects on CNS development for which inner city children are at an alarming high risk. To these must be added the effects of perinatal malnutrition, prematurity, understimulation, neglect, and abuse. All of these risk factors are associated with adverse outcomes (Mrazek and Haggerty 1994). Researchers typically focus on a single adverse event that leads to impairment in order to understand the mechanism. However, most studies indicate that inner city youth have been subject to multiple risk factors that are difficult to disentangle. Thus, maternal substance abuse is associated with poor perinatal care, low birthweight, neglect, intrafamilial violence, and greater risk for lead exposure. The concatenation of these risk factors conspire to restrict optimal development of the brain and to perpetuate social breakdown in the inner city.

The advances in our understanding of the fine features of brain development should be tightly coupled to the analysis of those factors that may impair these processes. First, it is not unreasonable to envision interventions that may extend over a critical or sensitive period to address or remediate early insults. Second, as hard scientific evidence becomes available, policy should be modified in response. For example, the appreciation of the neurotoxic consequences of lead resulted in the elimination of lead from gasoline. As demonstrated in a recent study (Brody et al. 1994), the blood lead levels in children were remarkably reduced between 1980 and 1991. In other words, prevention works.

Finally, we must remember the admonition "there are lies, damn lies, and statistics." While there is convincing evidence that genetic factors contribute to the determination of mental abilities, it is a serious error to conclude that the mental subnormality and behavioral pathology that ravages our inner city youth are primarily genetically determined. Those who recognize the remarkable plasticity of the brain must look for evidence of adaptability. The modest effects on behavior observed in recent studies, in which specific genes encoding for functionally important brain proteins have been knocked out, point to alternative and creative strategies of the nervous system to compensate for genetic malfunction (Coyle 1996). Conversely, the Human Genome Project is merely providing us with one element of the complex equation of nature and nurture that will assist us greatly in determining the role of environment in leading to the cognitive limitations that are disproportionately represented in inner city youth.

The focus on molecular and cellular mechanisms, however, should not distract us from the equally troubling issue of the personal narratives of these children that shape their self-image and moral development. Clearly, disruption of brain development limits choices and the ability to respond to adverse life experiences such as neglect, economic disparity, abuse, and academic underachievement. It should be hoped that the advances in neuroscience will be an agent for reducing the noxious environmental toxins that cognitively damage our youth, who face so many other personal and social challenges to achieving their potential.

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Shaping Forces Within the Early Parent-Infant Relationship

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ABSTRACT

In recent years, animal research has revealed a network of simple behavioral and biological processes that underlie the psychological constructs we use to define early social relationships. Hidden within the observable interactions of parent and offspring are sensorimotor, thermal, and nutrient-based events that have unexpected and widespread regulatory effects on infant behavior and physiology. The complex pattern of responses resulting from early separation in infant rats can be traced to the abrupt withdrawal of a number of discrete, independent regulatory processes which had been acting on individual components of the infant's physiology and behavior. These regulatory processes also appear to mediate long-term shaping effects exerted by early relationships, for example, on the vulnerability of the adult rat to hypertension and stress ulcer. In human development, early regulatory interactions may provide a bridge between biological and psychological processes in the development of our earliest mental representations.

For all mammals, during early development, the major source of the environmental component of the gene-environment equation is the mother, first in utero and then in the early postnatal period. This maternal environment is a predictable, heritable feature of the mammalian developmental plan, enabling evolution to select components of the infants' interaction with this early environment as well as genes to shape the infant's physical and behavioral development. Thus, as we look more closely at the mechanisms of early development, the maternal environment is emerging as a major source of novel processes that regulate the development of the young organism, even before birth. This chapter describes a few of these processes, focusing on those that

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involve behavioral interactions and the infant's first social relationship in particular.

Transgenerational Effects

Since the early maternal environment, unlike the genetic composition of the germ cells, is subject to changes induced by the experiences of the mother, this component of our developmental plan is subject to the inheritance of acquired characteristics. The processes involved in this form of transgenerational change can act prenatally or postnatally. Its mechanisms include placental transmission of active agents such as hormones, nutrients, drugs, and viruses as well as the stimulation of the fetus' and infants' sensory systems provided by the mother's behavior.

In the past decade, progress in developmental neurobiology has revealed a great many processes by which the early functioning of sensory and motor systems determine the fine structure of the brain during fetal and neonatal life. This early neural function, in turn, allows the brain of the fetus and newborn to be literally sculpted by its responses to its environment. One of the features of this environment is the behavior of its mother.

Evidence for the transgenerational effect of experiences occurring during the early life of the mother were first reported in carefully controlled rat experiments 30 years ago (Denenberg and Whimbey 1963; Denenberg and Rosenberg 1967). In Denenberg's experiments, the offspring (and even the grand offspring) of mothers that were handled daily by the experimenters during their infancy showed increased levels of emotionality on measures of exploration and defecation when, as adults, they were placed alone in a novel test arena. Through cross-fostering of pups at birth between mothers that were handled in infancy and those that were not, Denenberg was able to show that most of the effect was transmitted postnatally.

This is the only transgenerational effect thus far reported that is opposite in direction to the original effect of the early experience on the mothers. Handling of rats in infancy consistently produced decreased emotionality when these animals were simply tested as adults. Denenberg went on to show that when all mothers were given free access to larger and more complex housing conditions during the postnatal period, the transgenerational effect was altered so that it was now in the same direction as the effect on the mothers themselves, a reduction in offspring emotionality. The sensitivity of the maternal transmission effect to changes in the maternal environment during the postnatal period suggests a behavioral mechanism involving altered mother-infant interactions.

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But, as in all such transgenerational effects reported to date, the mechanism remains unknown.

We got involved in transgenerational effects through the urging of a graduate student in clinical psychology, Neil Skolnick, who was taking an elective in our laboratory. Sigurd Ackerman, Herb Weiner, and I (1975) had recently discovered that if rat pups were separated early from their mothers (at 15 days—early weaning), they developed a marked vulnerability to immobilization-induced gastric ulceration during early puberty, at 30 days of age. In our first transgenerational experiment (Skolnick et al. 1980), we let the early separated females grow up, become pregnant, and rear their litters normally. To our surprise, their litters were significantly more susceptible to stress ulceration than normal rats when tested at 30 days, even though they had not themselves been early weaned.

Next, we designed a replication study with cross-fostering of pups at birth between normal and early weaned mothers. Again, the offspring of mothers with a history of early weaning were significantly more vulnerable to stress ulcer, and the effect, unlike Denenberg's study, was entirely attributable to the prenatal period. Mothers that had been early separated as infants differed from the normal mothers during the postnatal period: they spent more time out of contact and less time nursing. But despite this behavioral difference, pups reared by these mothers showed normal stress ulcer vulnerability. The mechanism for this prenatal transmission of ulcer vulnerability is unknown, emphasizing how much we have to learn about this developmental period.

These studies, carried out during the 1960s and 1970s, represent the most far-reaching effects of early experience to have been reported under controlled conditions in the laboratory, and they support the speculations of social scientists and clinicians that historical events can alter behavior and vulnerability to stress over generations. They were carried out at a time when the new field of developmental psychobiology was defining itself, and scientists needed to convince themselves and others of the long-term importance of early experience. In the past 30 years, we have begun to learn enough about early developmental processes to know some of the possible mechanisms for these remarkable effects.

Prenatal Influences

Interest in behavior during the prenatal period was late to develop because the fetus was so hard to study and its behavior was thought to be made up of little more than primitive reflexes. With the advent of ultrasound imaging techniques and a method for briefly exteriorizing the rat fetus for experimentation, we have begun to learn something about this forgotten period in life and to realize how much behavior, including learning, begins in utero. The first book on fetal behavior appeared in 1988 (Smotherman and Robinson).

The first strong evidence for fetal learning came from studies on early voice recognition in humans that found that babies recognize and prefer their own mother's voice, even when tested as soon as possible after birth (de Casper and Fifer 1980). Bill Fifer has continued these studies in our department using an ingenious device through which newborns can choose between two tape- recorded voices by sucking at different rates on a pacifier rigged to control the tape player (reviewed in Fifer and Moon 1995). He has found that infants prefer human voices to silence, female voices to males, their native language to another, and their own mother to another mother reading the same Dr. Seuss story.

In order to obtain more direct evidence for the prenatal origins of these preferences (rather than very rapid postnatal learning), Fifer filtered the high-frequency components from the tapes to make the amplified voices resemble recordings made within the amniotic sac of pregnant women by hydrophone. This altered recording was preferred to the standard mothers' voice by newborns in the first hours after birth, a preference that tended to wane in the second and third postnatal days. Furthermore, there is now evidence that newborns prefer familiar speech and music sequences that they have been repeatedly exposed to prenatally (see Fifer and Moon 1995).

In a striking interspecies similarity, caeserean-delivered rat pups have recently been shown to discriminate and prefer their own dams' amniotic fluid over that of another dam (Hepper 1987). Newborn pups are known to require amniotic fluid on a teat in order to find and attach to it for their first nursing attempt (Blass 1990). Robinson and Smotherman (1995) directly tested the hypothesis that pups begin to learn about their mothers' scent in utero and have begun to explore neural substrates for this very early form of plasticity. They have been able to demonstrate one-trial taste aversion and classical conditioning in late-term rat fetuses, using intraoral cannula infusions and perioral stimulation. Taste aversions learned in utero were expressed in the free feeding responses of weanling rats nearly 3 weeks later. They went on to determine that responses to intraoral milk and to perioral stimulation depend upon kappa opiate and dopamine-1 receptors, respectively. It is apparent that these neural systems regulate simple state changes in the fetus, involving, in addition, altered response thresholds and heart rate responses.

These forms of fetal learning involving maternal voice in humans and amniotic fluid in rodents appear to play an adaptive role in preparing the infant for its first extrauterine encounter with its mother. They are thus the earliest origins found to date for the attachment to the mother that forms in the postnatal period and so characterizes and regulates the mammalian infants' behavior into the juvenile period.

Early Postnatal Learning and the Formation of Attachment

Although specific olfactory and/or auditory predispositions toward the infant's own mother may be acquired prenatally, after birth the infant enters a new world where contingent events, so important for more advanced forms of learning, are now occurring with great frequency. Regina Sullivan, Steven Brake, and I (1986) showed that associating an odor with simulated licking of the pup, after just a few repetitions, resulted in the pup learning to select, approach, and remain close to that odor. Several different kinds of tactile stimulation, even tail pinch and mild electric shock, also induced preferences for the odor associated with them during the first week of postnatal life. But such strong and seemingly aversive tactile stimulation ceased to induce preferences and then began to induce avoidance responses during and after the second postnatal week, demonstrating a sensitive period for the formation of positive associations reinforced by intense tactile stimulation. The odor association sequences in the first postnatal week not only produced olfactory preferences, but the specific odor also came to elicit increased active huddling, probing, and pawing behavior and increased time spent in contact with an inert target animal scented with the odor (Sullivan, Brake, et al. 1986). Thus, the odor came to arouse the same behaviors originally produced by interaction with the mother.

This rapid learning process resembles imprinting in birds and reminds one of clinical observation of strong attachments of children to abusive parents: it occurs during an early sensitive period, does not require standard reinforcing events, and accommodates even intense levels of stimulation as reinforcing. It produces responses that are highly specific to an identifying maternal olfactory cue. This cue elicits an arousal state and becomes an incentive in a motivational system that ensures close proximity of the infant to the mother. The degree of specificity conveyed by this early learning results in rat pups at 10 days of age being able to discriminate and prefer their own mother or their own littermates from another lactating dam or its littermates (Hepper 1986*a*, 1986*b*).

Early olfactory learning of this sort has become a model system for neuroanatomical studies that have established the existence of a distributed memory system involving the amygdala, the hippocampus, and thalamocortical systems as well as the olfactory bulb and cortex (Wilson and Sullivan 1994). Norepinephrine appears to play a dual role in this learning, enhancing olfactory system responsiveness during training and permitting later consolidation. Dopaminergic, serotonergic, glutamatergic, and GABA receptors have also been implicated.

Maternal Regulation of Infant Systems

Soon after birth, prenatally acquired perceptual biases, stimulus-guided tactile responses, and associative learning create a powerful behavioral control system through which the infant maintains close proximity to its mother. Another important attribute of attachment, by which the emotional tie of the infant to its mother is inferred, is the response to separation. This is generally supposed to be an integral part of the proximity-maintenance system, one that represents the affective expression of its motivational nature. Thus, it has been held that the degree or strength of attachment may be inferred from the intensity of the response to separation, and the response itself represents a full expression of the attachment behaviors in the absence of their goal object. Experiments in our laboratory have led us to a very different view, in which the processes underlying attachment and the response to separation are seen as separate and distinct early in life (Hofer 1995).

The response of infant rats and primates to maternal separation has been found to involve a complex pattern of changes in a number of different behavioral and physiologic systems (Hofer 1994). We found that this pattern was not an integrated psychophysiological response, as had been supposed, but the result of a novel mechanism. During separation, each of the individual systems of the infant responded to the absence of one or another of the components of the infants' previous interaction with its mother.

Providing one of these components to a separated pup, for example maternal warmth, maintained the level of brain biogenic amine function underlying the pups' general activity level, but had no effect on other systems, for example, on the pups' cardiac rate, which fell 40 percent after 18 hours of separation, regardless of whether supplemental heat was provided. The heart rate, normally maintained by sympathetic tone, we found was regulated by maternal provision of milk to neural receptors in the lining of the pup's stomach. By studying a number of other systems, such as those controlling sleep-wake states, activity level, vocalization, and blood pressure, we concluded that in maternal separation, all the regulatory components of the mother-infant interaction were withdrawn at once, yielding a pattern of increases or decreases in level of function of the infant's systems, depending upon whether the particular system had been upregulated or downregulated by the previous mother-infant interaction.

Other investigators, using our approach, have discovered other such developmental regulators (Kuhn et al. 1990) and shown that they can also be found in human infants. For example, repeated, vigorous tactile stimulation prevents the fall in growth hormone that follows maternal separation in rat pups (Kuhn and Schanberg 1991). When administered 3 times a day for 15 minutes over 2 weeks to low birthweight prematurely born babies, a stroking regimen significantly increased weight gain, head circumference, and behavior development test scores, with effects discernible many months later (Field et al. 1986).

The vocalization response of the infant, traditionally viewed as an attachment behavior (bringing the mother to the pup from a distance) and as an expression of the emotional response to disruption of the attachment bond, appears instead to represent the loss of multiple regulators normally present when the infant is in contact with its familiar littermates or mother. Isolation calling in young rats has been shown to be mediated by the same central neuromodulator systems implicated in human anxiety states (reviewed in Hofer 1996). Thus, contact with familiar companions appears to operate as a simple form of regulation of the infants' emotional state. When warmth and specific olfactory and tactile stimuli are combined in an artificial surrogate, the infants' isolation calling rate is reduced as effectively as if it were a familiar littermate or its own mother.

These studies provide us with a new way to understand separation responses that does not depend upon attachment. We have found regulatory processes hidden within the observable interactions of mother and infant (such as contact, warming, grooming, a variety of tactile interactions, and nursing) that maintain the level of function in infant physiology and behavior within certain limits. Over time, these regulatory interactions shape the development of infant systems, and different qualities or types of interaction can have different long-term effects on infant physiology or behavior through a different intensity, mix, or balance of the component regulatory interactions (reviewed in Hofer 1994). This is the kind of long-term shaping influence of the maternal relationship that could underlie some of the transgenerational effects described in the first section of this chapter. The next section describes efforts to explore these possibilities.

Adult Trait Transmission

In 1974, two Czech investigators reported the results of a study in which they cross-fostered at birth the offspring of two different strains of rats produced in their lab by selective breeding for high and low aggression toward mice (Flandera and Novakova 1974). They found that the different traits in the offspring were acquired through the postnatal maternal environment, rather than the genotype of the pup as determined by the strain of the biological mother. Since the pups never observed adults interacting with mice, the different traits were evidently transmitted by the mothers during interaction with their foster pups prior to weaning. Low-aggression strain pups fostered to high-aggression strain dams showed high levels of aggression (65 percent) both at 30 and 90 days of age.

Conversely, pups from the high-aggression strain, when cross-fostered to low-aggression strain mothers, showed minimal levels of mouse killing (12.5 percent) as adolescents (30 days). However, their genes gradually became expressed as the offspring developed increased levels of aggression by 90 days of age (50 percent). This study is a good example of how selective breeding can exert its effect indirectly, through altering maternal behavior toward the pups as well as through more direct effects on offspring behavior.

A more recent example is the case of the spontaneously hypertensive strain of rats (SHR) and their Wistar Kyoto (WKY) progenitor control strain. It has been shown that fostering of the young of the SHR strain at birth to WKY mothers normalizes their adult blood pressure (BP) but the low BP of WKY rats was not increased by fostering them at birth to SHR dams (McCarty et al. 1992). The BP of both strains was unaffected by the fostering manipulation itself within strains, and cross-fostering within the 3 weeks following birth showed that the sensitive period for the normalization of BP lay within the first or second postnatal weeks.

We have explored the possible mechanisms for such an effect in a series of studies (reviewed in Myers et al. 1992). First, we found that laboratory SHR rats showed more rapid, but less efficient, retrieval of pups than WKY rats in a standard test of maternal behavior. This led to an extensive observational study of maternal behavior throughout the preweaning period in the two strains. From this study, we learned that although members of each of these inbred strains were genetically identical, there was significant interlitter variability both in adult BP and in maternal behavior within each strain as well as an overall difference in both variables between the two strains.

Three maternal behaviors accounted for most of the variability in adult blood pressure, both between litters and between strains. Pups of mothers that showed more of these behaviors had higher BP as adults. By looking carefully at the components of maternal behavior that were most highly correlated with adult blood pressure of the offspring, Myers and Shair made an educated guess that led them to the next chapter in the story. Since milk letdown during nursing occurred during one of the behaviors linked to the adult trait, we measured blood pressure of the pups during nursing and found a sudden transient rise in blood pressure of 50 percent in response to maternal milk letdown. This was a greater increase than pups showed during any other activity.

Through a series of analytic experiments, Myers found that this surge in blood pressure was caused by a major increase in the autonomic neural activity controlling blood vessel tone throughout the body, and Shair and I (1993) found that it was triggered by contact of the milk with sensory nerve endings on the tongue and in the throat. Myers has gone on to show that the rate of weight gain during a critical period of nursing is a powerful correlate of adult blood pressure and that experimentally increasing the level of milk letdown events during this 4-day period by a temporary reduction in litter size, significantly increased adult blood pressure of offspring. The importance of these studies was further increased when Myers found that human infants also show major increases in blood pressure in response to maternal milk letdown.

Thus, in these most recent experiments, we have returned to the questions raised in the first part of this chapter and can now begin to see how qualitative differences in behavior in the parental generation may powerfully influence the expression of genotype in their offspring. It appears that we have a great deal still to learn about the forms and mechanisms of plasticity arising within early social relationships.

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Early Emotional Development: Integrative Perspectives From Longitudinal Study

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Developmental plasticity has typically been thought of as the rechanneling of a developmental pathway that involves both structural and functional changes. This chapter illustrates three major principles emerging from recent work. These principles deserve emphasis in the midst of our challenge of integrating increasing knowledge from molecular genetics and the developmental neurosciences with knowledge of behavioral development. Illustrations of the principles come from the longitudinal study of emotion expression during the child's second year.

A first principle concerns the dynamic changes in genetic and environmental influences on behavior over time. In addition to environmental change, our notions of plasticity need to include processes of genetic change, and genetic change may come about as a result of changes in the environment. A second, related, principle concerns the importance of context in behavioral organization: genetic and environmental influences may differ as a function of the context in which behavior occurs. A third principle concerns developmental transitions. There are certain times when major changes in person/environment relations may provide nodal points for understanding developmental plasticity. At such times, new resources are deployed by the child, emotional changes are likely, and socioenvironmental responses are reconfigured.

The first two principles are illustrated by analyses from the MacArthur Longitudinal Twin Study, which uses a behavioral genetics approach to model genetic and environmental influences with respect to change and continuity in a way that can point to future directions for more targeted research. The third principle, that of developmental transitions, is illustrated by two other longitudinal studies focusing on socioemotional changes that occur with the onset of walking and the onset of multiword speech.

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The chapter concludes with a statement about emotions and their development. In the past 20 years, we have seen changing perspectives resulting from our research on emotions. During the next 20 years, genetics and the brain sciences will clarify a number of key issues.

Empathy: Components, Change, and Context

Empathy is an emotional response that can also be considered an aspect of moral development. It begins in the second year of life when children display integrated patterns of concern for others in distress. Zahn-Waxler, Radke-Yarrow, and colleagues (Radke-Yarrow and Zahn-Waxler 1984; Zahn-Waxler, Radke-Yarrow, et al. 1992) developed an experimental paradigm in which mothers and testers could systematically simulate distress and evoke such responses from children, eliciting behaviors for systematic developmental assessment. Such techniques for assessment of empathy also lend themselves to studies of individual differences across development and opportunities for understanding genetic and environmental influences.

The MacArthur Longitudinal Twin Study, whose method is documented elsewhere (see Emde et al. 1992; Plomin et al. 1990; Plomin et al. 1993) used the Zahn-Waxler et al. observational approach for assessing empathy during the child's second and third years. Five occasions of observation were aggregated at each age. Table 1 illustrates differences in correlations between monozygotic (MZ) and dizygotic (DZ) twins at 14 and 20 months. It also shows the value of breaking down this emotional response into observed cognitive (hypothesis-testing), emotional arousal (empathic concern), and behavioral (prosocial acts) components. Significant heritability is estimated by differences between MZ and DZ correlations, using the regression model of DeFries and Fulker (1985), and is indicated in the table by underlined values. One can also

TABLE 1.—*Empathic responses of 14- and 20-month-old twins to simulated adult distress*

[Intraclass correlations]

	14 months		20 months	
Variable	MZ	DZ	MZ	DZ
Responses to distress				
Hypothesis testing	.28**	.08	.16	.15
Empathic concern	.29**	-.05	.30**	.09
Prosocial acts	.43**	-.14	.09	.20
Maternal report	.86**	.69**	.81**	.63**

Source: Adapted from Zahn-Waxler, Robinson, and Emde 1992.

**p<.01; underlined twin correlations correspond to significant estimates of heritability; N=94 MZ, 90 DZ pairs observed; N=86 MZ, 84 DZ pairs for maternal report.

see the different pattern of genetic influences for empathy components at 14 and 20 months.

Findings at 24 and 36 months of age also indicate changes in patterns of genetic influences on empathy components. Recent analyses revealed another striking effect, namely, one resulting from the context of measurement. Table 2 shows the pattern of twin correlations aggregated separately for the two occasions when a research assistant simulated distress versus two occasions when mother simulated distress. Again, significant differences in twin correlations indicating genetic influences are underlined, and one can see that they are prominent, with MZ correlations higher, when the research assistant was distressed. On the other hand, when the mother was distressed, genetic influences were not prominent; instead, shared environmental effects are prominent. Significant influences from the shared environment are indicated by italicized values where both MZ and DZ twin correlations are elevated.

TABLE 2.—*Empathic responses of 24- and 36-month-old twins to simulated adult distress*

[Intraclass correlations]

	24 months		36 months	
Variable	MZ	DZ	MZ	DZ
Examiner simulating distress				
Responses to distress				
Hypothesis testing	.39**	.13	.61 **	.17
Empathic concern	.36**	-.11	.33**	-.07
Prosocial acts	-.10	.15	.30**	.13
Mother simulating distress				
Responses to distress				
Hypothesis testing	_.52**_	_.50**_	_.29**_	_.25*_
Empathic concern	_.51**_	_.55**_	.22	.16
Prosocial acts	.37**	.24	.33**	.24

** p<.01; * p<.05; underlined twin correlations correspond to siqnificant estimates of heritability; pairs of twin correlations in italics correspond to significant estimates of shared environmental influences.

How we can explain the difference in results depending on who simulated distress? One interpretation of this difference is that socialization influences on empathy, shared within a family, are of major importance when mother shows distress but are not when the research assistant does. The context of a child's experience within a meaningful caregiving relationship appears to make a difference. Clearly, the context for observed responses and their measurement are important considerations in understanding when genetic and environmental influences may operate.

Reactions to Restraint and Maternal Reports of Anger-Related Expressions

Anger expressions in infancy have been described in a variety of different measurement contexts, including arm restraint (Fox 1989; Stenberg and Campos 1990), during stranger approach (Fox and Davidson 1988), during play (Goodenough 1931), during socialization experiences with parents (Radke-Yarrow and Kochanska 1990), and during frustration of needs and cookie withdrawal (Klinnert et al. 1984; Stenberg et al. 1983). Controversy remains, however, as to the extent to which anger in infancy can be regarded as an integrated, discrete, biologically based response system versus the extent to which anger responses depend on the context of measurement and are thus better typified in terms of component responses.

Recent analyses from our MacArthur Longitudinal Twin Study provide interesting findings with respect to the importance of context as well as change over time. Observed restraint protest was aggregated across four measurement conditions (the toddler being held while measured, being jacketed in the home, being jacketed in the laboratory, and being restrained during an electrode placement procedure). Maternal reports of anger-related expressions were quantified from systematic interview and included rating scales of angry outbursts, initiation of fights with the co-twin, and global reports of anger frequency. All of these measures, individually, showed moderate stability across the age periods of 14, 20, and 24 months, with correlation levels in the 0.3 to 0.5 range. On the other hand, phenotypic correlations among these measures were remarkably low (in the zero to 0.2 range), indicating that we had little justification for composing a construct of an integrated anger-related response across these measures.

Still, genetic analyses were revealing. Univariate twin correlations at each age indicated genetic influence for all of these measures and substantial common environmental influences as well. We therefore proceeded to longitudinal model-fitting analyses according to the method described in Cherny et al. 1994. Parsimonious models for genetic and common environmental influences that are unique at each age and that go across age are presented in figure 1 for "restraint protest" and in figure 2 for "initiates physical fights."

The figures illustrate different, but coherent, patterns for each of these measured responses. For restraint protest, a genetic factor is present from 14 months that contributes to phenotypic stability at all three ages. The picture is one of genetic continuity. On the common environment side of figure 1, new influences can be seen to come in at each age and, in addition, moderate influences continue from each of the first two factors (at ages 1 and 2) across time.

For "initiates physical fights," the picture is one of genetic change as well as continuity. A two-factor genetic model is needed to explain the data, with a new genetic influence at 20 months and with influences going across ages from

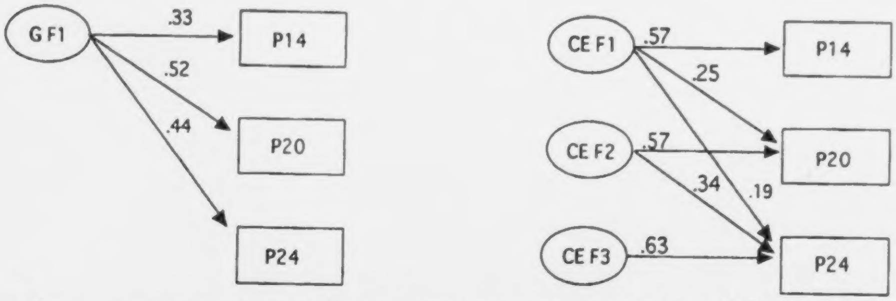

Figure 1 Longitudinal model-fitting: Restraint protest. Genetic (G) and Common Environment (CE) Factors contributing to Phenotype (P) at 14, 20, and 24 months.

both 14 and 20 months. On the other hand, for the common environment, the parsimonious model indicated new influences on the phenotype at each age, with no influences going across age that would indicate continuity.

Different pictures of genetic and environmental influence are revealed by model fitting for "angry outbursts" and global reports of anger frequency across these same age periods. At this point, therefore, we have results that are context specific. Although there are coherent patterns of developmental change within particular responses over time, we cannot conclude that we have any evidence for a general construct of anger propensity that goes across context.

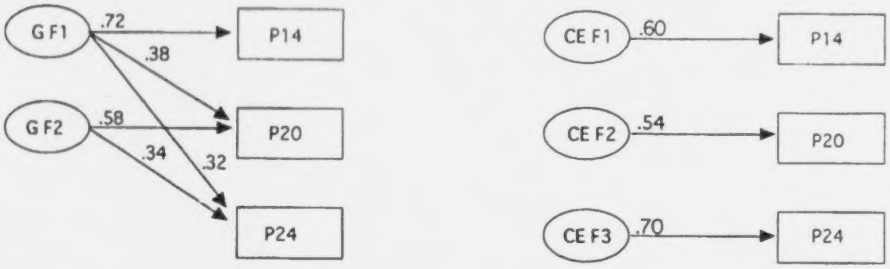

Figure 2 Longitudinal model-fitting: Initiates physical fights. Genetic (G) and Common Environment (CE) Factors contributing to Phenotype (P) at 14, 20, and 24 months.

Developmental Transitions: Walking Onset

All development is characterized by change within individuals over time. There are certain times in development, however, when changes are not only more rapid, but they have other qualities. They are pervasive, occurring across multiple domains of functioning, and they are enduring. Moreover, they also involve changes in person-environment relations, including new roles of the individual within the family.

Over the course of time, we have studied a number of these developmental transitions in early childhood. Here, we provide the example of walking onset. The developmental transition to walking had been observed to be associated with a change of affectivity and autonomy (Mahler et al. 1975) but had not been studied systematically. Our study design of this transition involved longitudinal home and laboratory observations of infants who were recruited prior to walking, at 8 months (Biringen et al. 1995). After baseline study, a second observation was triggered by the onset of consolidated walking, when the child would not just take a single step but would be able to walk halfway across the room in order to do something.

For this second observation, the one-half of the study infants who had begun walking in this way was paired for purposes of analysis with the other half of the study infants who were not walking. Thus, we recruited enough of a sample to enable us to age-match infants, one-half of whom would be walking and one-half not walking. We then continued longitudinal study to include the transition to walking in the second group of infants so that we could examine postwalking for both groups. We should emphasize that this was a nonclinical sample with no extremes of either very precocious walkers (beginning prior to 8 months) or delayed walkers (beginning after 14½ months).

Our hypotheses for this study, following the work of Mahler et al. (1975), were that this developmental transition would be associated with an increase in positive emotionality, along with an increase in autonomy, exploration, and willfulness. The latter would be evident by the child's persistence of activity and expression of negative emotions when maternal prohibitions took place. To our surprise, we found evidence for the above hypotheses with respect to earlier walkers and not for later walkers. In other words, the hypothesized features of affective reorganization involving the infant, the mother, and the dyad, occurred for those infants who began consolidated walking earlier, approximately between the ages of 9 and 12 months of age, but not for those who began walking between 12 and 14 months of age.

Interestingly, there was a suggestive difference in temperament between the groups of earlier (n=23) and later (n=23) walkers. The earlier walkers were characterized by more "distress to limitations" on the Infant Behavior Questionnaire of Rothbart (1981) at all three occasions of measurement—before, during, and after walking onset. This subscale of temperament indexes the response of the infant to being restrained during everyday routines, according to mother's report. The implications of the temperament findings await further research.

Our results indicate the importance, not only of a shift in person-environment relations during this developmental transition, but also of timing variations; indeed, timing may be important for the onset of other transitions. Currently, our group is completing a study of the developmental transition to multiword speech toward the end of the child's second year. Our interests are in the changes in the emotional communication system during the onset of this major milestone in language acquisition.

Profiting from what we learned in the walking onset study, we preselected two groups of infants according to early language measures, with one group expected to be early talkers and the other expected to be later talkers. We are tracking them from 13 to 30 months to look at changes in the organization of affect and communication.

Conclusion

Over the past 20 years, there have been major changes in the way we view emotions. Whereas we used to view emotions largely as reactive, intermittent, and disruptive events, we now take an organizational perspective, viewing emotions as active, ongoing, and adaptive processes. Emotions are motivational and serve the function of communication between individuals. And the principle of regulation is central.

Another change in our view involves an appreciation of complexity. Whereas we used to regard a set of basic emotions as being hierarchically nested in some straightforward way, we now regard emotions as processes that have meaningful components and configurations, with an ordering that is not likely to be nested in any simple way but is often nonlinear and complex. Related to this is our change toward a relational view. Whereas emotions used to be studied in an isolated, mechanical way, we now appreciate that processes of emotion involve person-environmental relations of adaptive significance (Campos et al. 1989; Lazarus 1991). Emotions are constructs that can be understood only in terms of goals of the individual in relation to the environment of objects or persons.

The issues that arise with respect to all of our more recent views are likely to be clarified by accelerating advances in molecular genetics and the neurosciences. First, types of dysregulation in emotional patterning are likely to be clarified by discoveries of genetic aberrations and deficiencies found at molecular levels, and their consequences for maladaptive functioning will become clear also. Correspondingly, the nature of regulatory processes involving adaptive functions will be better understood. Moreover, it may be that processes dealing with individual strength and mental health (i.e., regulation) may be different in kind from processes dealing with emotional dysregulation in illness—and this may have consequences for intervention.

Second, in terms of complexity, we will understand more about the genetics of components and their configurations. It seems likely that new emotional clusters and regulatory processes will emerge. From the relational view, we will come to understand more about the context specificity of components of emotional predispositions and the situations that elicit them. Genetic potentials are activated only in particular environments and, in turn, are influenced by them. Thus, we will discover more and more about the contexts that are specific for mental health as well as for mental illness.

Finally, development introduces increasingly organized complexity and change within individuals over time. New configurations of emotional processes will appear with increasing complexity. These will have different phenotypic forms as well as new genetic and environmental influences. With increasing complexity in person-environment relations, we remind ourselves that many of these configurations will occur in the context of intimate social relationships. Correspondingly, one will expect that genetic and environmental influences will continue to vary throughout the lifespan in the context of changing intimate social relationships.

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Maladies of Love—An Evolutionary Perspective on Some Forms of Obsessive-Compulsive Disorder

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ABSTRACT

At the level of subjective experience and behavior, the early phases of romantic love and early parental love share much in common: perception of an altered mental state, intrusive thoughts and images associated with a heightened awareness of the other, and a complex behavioral repertoire aimed at eliciting a reciprocal response. In many instances, these altered mental states lead to the same outcome—the formation of intimate interpersonal ties. Although romantic and early parental love are clearly distinct phenomena, an examination of those elements shared in common may provide a useful vantage point for considering the evolution and neurobiology of love and its range of normal and psychopathologic outcomes. We selectively present portions of the ethnological and psychological literature for both romantic love and early parental love before turning to an examination of the available neurobiological data on central oxytocin pathways and their role in the initiation of pair bonding and early parental behaviors in many mammalian species. This is followed by a consideration of various forms of psychopathology that contain either shared behavioral elements or neurobiological substrates. We conclude with the view that it is likely that certain adaptive "sets" of human mental states and behaviors are evolutionarily conserved and that this conservation is reflected in our genetic makeup, the functional neurobiology of our brains, our behavior during related developmental epochs, and our vulnerability to certain forms of psychopathology, including autistic disorder, obsessive-compulsive disorder, and drug dependence, among others.

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Dennett (1995) has likened Darwin's theory of evolution to a "universal acid" that can eat through any of our most cherished views. Traditional concepts concerning why some of us may become "mentally ill" are no exception. This brief note reports on the effect of applying this acid to existing psychiatric nosologies. Our initial focus is on obsessive-compulsive disorder and related forms of psychopathology, but once having applied the acid, it has proven difficult to slow its progress.

We first became interested in this perspective because of an unexpected, and as yet unconfirmed, finding of elevated levels of oxytocin in the cerebrospinal fluid of a sizable subgroup of patients with obsessive-compulsive disorder (Leckman et al. 1994*a*, *b*). Oxytocin is best known for its peripheral effects in parturition and nursing. More recently, central oxytocin pathways have been strongly implicated as one of the neurobiological substrates of the initiation of maternal behaviors in mammals (Insel 1992*a*). This led to a reframing of our initial question and a tentative hypothesis that the intrusive thoughts and bothersome rituals of obsessive-compulsive disorder are in some way related to the intense preoccupations and loving habits that envelop early parenthood. This chapter begins by taking stock of this hypothesis using data from an ongoing study of expectant parents.

Preoccupations and Habits Reported in Early Parenthood

Empirical studies of the early parent-child relationship have usually centered on the infant. The mental state of the parent is usually of secondary concern unless something untoward occurs in the parents' care of the infant. Winnicott (1956), a British pediatrician and psychoanalyst, drew attention to the normal preoccupations of new parents and pointed to their likely value to enhance the mother's ability to sustain a nurturing environment that can meet the physical and psychological needs of her infant.

Being unaware of any empirical studies that have examined this psychological state, two of us in collaboration with King and Cohen at Yale, Feldman at Bar-Ilan University in Israel, and Evans at the University of New Orleans set out to chart this territory. For this purpose, we crafted a three-part parental interview, "Parental Inventory of Thoughts and Actions," that was based in part on the Yale-Brown Obsessive-Compulsive Scale (Goodman et al. 1989*a*, *b*). Thus far, 18 parents have completed interviews at 8 months gestation, 2 weeks postpartum, and 3 months postpartum.

An interim analysis of these data has confirmed the intense preoccupation of parents during this period. Mothers reported on average that their minds were "occupied" with thoughts about their baby from 7 to 12 hours per day, and that at 2 weeks postpartum, many parents could not go more than 5 minutes without having thoughts concerning the child (figure 1). As we predicted, this preoccupation was most intense in the days surrounding the delivery. The content of many of these thoughts was fairly predictable and directed at the comfort and physical well-being of the baby. Others were reminiscent of the preoccupations of romantic love—the perfection of the child and longings to achieve a deep, nearly indescribable, emotionally satisfying reciprocity.

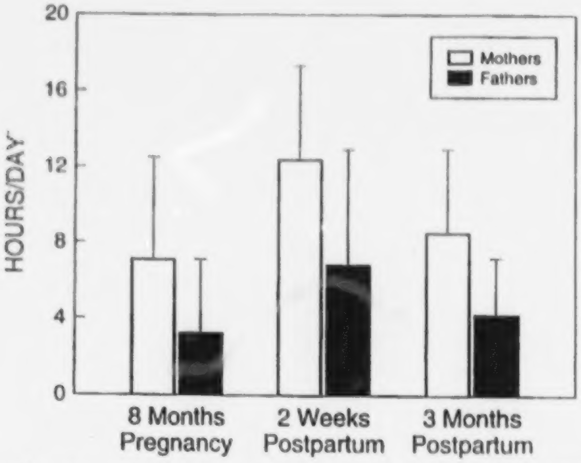

Figure 1 Parental estimates of the amount of time that their minds were "occupied by thoughts about their baby" at 8 months of pregnancy, 2 weeks postpartum, and 3 months postpartum.

As in romantic love, these reveries of being "at one" with the other were frequently interrupted with intrusive worries about something bad happening that would compromise the relationship. In the case of new parents, these negative thoughts included worries about the baby—an illness, a fall, or worse occurring because of the parents' neglect or failure to read the infant's signals correctly. Other parental fears concerned their own health and well-being.

In a fashion similar to that encountered in patients with obsessive-compulsive disorder, the new parents also described a range of harm-avoidant behaviors associated with these intrusive thoughts, such as checking the infant repeatedly even when they rationally knew the baby was fine. Some of these harm-avoidant behaviors were evident prior to delivery and included special preparations for the nursery and extensive cleaning projects in the home.

Natural Selection, Parental Preoccupations, and Harm-Avoidant Behaviors

Viewed from an evolutionary perspective, it seems self-evident that early parenting skills would be subject to intense selective pressure. For our genes to self-replicate, the progeny must survive. The first year of an infant's life is fraught with mortal dangers. The physical and psychological environment must be shaped to ensure the infant's safety as well as healthy, adaptive growth and development.

Another implication, at first surprising, is that it is unlikely that nature has left parenting skills to chance. There are undoubtedly genes, perhaps even "families" of genes, that to some degree shape parental behavior. Natural selection in its mindlessness has probably subtly sculpted parental mental states, as well as behaviors, from gene products and neurobiological systems over the millions of years of mammalian and human existence. Although this process was conditioned by accidents and coincidences at every turning, the end result is for our species, anyway, one of the wonders of nature. Could we have designed this pattern of parental preoccupations and behaviors any better—seeking reciprocity to establish an internal equilibrium, to entrain the infant's rhythms of activity and sleep, and to create the foundations of interpersonal communication and language? On the level of behavior, it makes perfect evolutionary sense to pay close attention to the family's hygiene and safety.

A Hypothesis Concerning Some Forms of Obsessive-Compulsive Disorder

Mayes, Insel, and I have hypothesized that the evolution of the mental and behavioral states associated with early parenthood (and related states associated with the formation of monogamous pair bonds) has also produced a vulnerability to various forms of psychopathology, including obsessive-compulsive disorder.

Obsessive-compulsive disorder is a phenotypically heterogenous condition characterized by intrusive thoughts and preoccupations, rituals, and compulsions (American Psychiatric Association 1994; Rasmussen and Tsuang 1986). It can become a chronic disabling condition in which the individual repeatedly experiences the sudden intrusion into consciousness of unwanted thoughts or images and urges to perform seemingly senseless acts over and over again. These intrusive mental images that beseige the consciousness often involve sexual or aggressive ideas that the individual regards as repugnant and morally reprehensible. An individual can become totally preoccupied with these unpleasant and unwanted worries. In other instances, the individual is suddenly aware that something in a familiar environment does not look or feel or sound "just right" (Leckman et al. 1994*c*, 1995).

Compulsions are repetitive acts that are often performed a certain number of times or according to certain private rules that the individual is driven to complete, even though the act is perceived as excessive and/or senseless. Compulsions are often preceded by an urge that is recognized to be of internal origin that bears some relationship to obsessional worries (Rachman and Hodgson 1980). The most common compulsions are concerned with fears of contamination leading to hand washing or other grooming behaviors or some "pathological" doubts leading to repeatedly checking something to prevent some catastrophe, for example, repeatedly checking the stove to ensure that a fire does not start inadvertently. Despite potential embarrassment, performance of compulsive washing and checking is frequently associated with a measurable reduction in the subjective discomfort generated by the obsessional worries (Hodgson and Rachman 1972; Rachman et al. 1976; Röper et al. 1973; Röper and Rachman 1976). In both obsessions and compulsions, patients frequently report their efforts to resist mentally these unwanted ideas, images, and urges to act.

The evidence linking obsessive-compulsive disorder and the evolutionary conserved mental and behavioral states associated with early parenting is not compelling at present. It rests largely on phenotypical similarities, indirect data from natural history studies, and preliminary neurobiological data concerning the neuroanatomical circuits and neuroendocrine systems implicated in obsessive-compulsive disorder and paralleled, in part, by those implicated in animal models of parenting.

Similarities Between Obsessive-Compulsive Disorder and Mental States and Behaviors of Early Parenthood

Phenotypically, many obsessional thoughts with violent, aggressive, or sexual themes can also be described as obsessional worries about separation and what would happen if harm befell a close family member. The heightened sense of responsibility found in many obsessive-compulsive disorder patients (Rachman 1993) is also reminiscent of the increased feelings of commitment and responsibility that attend parenting. For example, as we noted in our study of expectant parents, it is not uncommon for parents to ensure that the home environment that the new baby will be entering is safe, secure, and free of contaminants by engaging in extensive cleaning or remodeling projects in the nursery. Similar behaviors, absent the baby, are commonplace among obsessive-compulsive disorder patients with contamination fears and cleaning compulsions. In many of these cases, obsessive-compulsive disorder is an illness that involves close family members as they are caught up in the patient's need to perform washing or cleaning rituals. In childhood-onset obsessive-compulsive disorder, this role frequently falls to one of the parents, as they assist their child or adolescent in one or another bathroom washing or cleaning rituals. In adult obsessive-compulsive disorder, parents or their spouse may actively participate in the rituals.

Linking obsessive-compulsive disorder with these normal periods of heightened preoccupation may begin to explain the intensity of the anxiety that some obsessive-compulsive patients experience when things are not just right at home. For most obsessive-compulsive disorder patients, the sense that things are not right is much more likely to occur at home and can prompt a range of checking behaviors. The senselessness of checking and rechecking a door or window for hours on end might begin to make some sense if one were caught in some ancient and conserved behavioral program to protect one's family from some immediate external threat.

Circumstantial Evidence From the Natural History of Obsessive-Compulsive Disorder

Perhaps the strongest piece of circumstantial evidence linking obsessive-compulsive disorder and conserved patterns of early parental behaviors concerns the increased risk of onset or exacerbation of obsessive-compulsive symptoms during pregnancy and the immediate postpartum period (table 1). Indeed, 11 to 47 percent of women reported that their obsessive-compulsive symptoms first appeared during pregnancy and the weeks following delivery (Bultolph and Holland 1990; Ingram 1961; Lo 1967; Neziroglu et al. 1992; Pollitt 1957). Most of these cases began during late pregnancy or a few days to 2 weeks after delivery. Other frequently reported precipitants included sexual or marital problems (12.5 to 46 percent) and the illness or death of a near relative (11 to 15 percent) (Ingram 1961; Lo 1967; Pollitt 1957)—all of which may point to the importance of separation and attachment issues in obsessive-compulsive disorder.

TABLE 1.—*Natural history studies of obsessive-compulsive disorder reporting an association with pregnancy and the postpartum period*

Study	N	Results
Pollitt 1957	150	At least 11% (10/93) of women had onset during pregnancy or childbirth.
Ingram 1961	89	47% (9/19) of women with children had onset during pregnancy or at childbirth
Lo 1967	56	12.5% (3/24) of women had onset during pregnancy or delivery
Bultolph and Holland 1990	60	36% (14/39) of women had onset during pregnancy or during the postpartum period. Another 28% (11/39) reported worsening of symptoms.
Neziroglu et al. 1992	106	39% (23/59) of women with children had onset during late pregnancy or during postpartum period.

Neurobiological Data

Two lines of evidence converge and suggest that the same or at least overlapping neurobiological systems are involved in both obsessive-compulsive disorder and the initiation and maintenance of maternal behaviors. One line of neurobiological evidence concerns the cortico-striato-thalamocortical (CSTC) circuits implicated in obsessive-compulsive disorder, which also appear to be required for normal mother-offspring interactions. The other line focuses on the apparent role of central oxytocin pathways in initiating maternal behavior and the preliminary data implicating oxytocin pathways in the pathobiology of obsessive-compulsive disorder.

The Role of Specific Cortico-Striato-Thalamocortical Circuits

During the past decade, there has been considerable progress concerning the neuroanatomical substrates involved in obsessive-compulsive disorder. The brain areas most frequently identified by in vivo neuroimaging studies are the orbitofrontal cortex (OFC), the anterior cingulate cortex, and the head of the caudate nucleus (Insel 1992). The OFC, which maintains extensive connections with the amygdala and hypothalamus as well as projecting to the basal forebrain and autonomic centers in the brainstem, has consistently been shown to have increased rates of glucose utilization in unmedicated obsessive-compulsive disorder patients (Baxter et al. 1987; Nordahl et al. 1989; Rausch et al. 1994; Swale et al. 1991; Swedo et al. 1992).

The anterior cingulate cortex has been strongly implicated in the pathobiology of obsessive-compulsive disorder by the encouraging results of neurosurgical procedures directed at this structure and related fiber tracts (Bingley et al. 1977; Fodstad et al. 1982; Jenike et al. 1991). The head of the caudate nucleus and the closely associated nucleus accumbens, which receives input from both the OFC and the anterior cingulate cortex, has also been implicated in obsessive-compulsive disorder. Specifically, two in vivo neuroimaging studies have found that changes in the glucose utilization in the head of the caudate nucleus are correlated with treatment (pharmacological or behavioral) response (Baxter et al. 1992; Schwartz et al. 1996).

The OFC, anterior cingulate cortex, and the head of the caudate are functionally interrelated limbic structures that are part of CSTC circuits. These circuits are likely to channel and subchannel emotionally laden information (Alexander et al. 1986; Parent and Hazrati 1995*a*, *b*). Baxter et al. (1992) and others (Modell et al. 1989; Pitman 1989; Rapoport and Wise 1988) speculated that portions of these circuits are hyperactive or "disinhibited" in obsessive-compulsive disorder, creating a "self-reinforcing loop that is difficult to break." More specifically, Baxter et al. (1992) suggested a disinhibition of the thalamocortical portions of these circuits due to an inadequate "filtering" of OFC "worry" inputs to the caudate nucleus and a subsequent decrease in the pallidal inhibitory output to the thalamus. Insel (1992*b*) offered the contrasting view that the hypermetabolic state observed in the OFC may be the product of the individual's resistance to the obsessive-compulsive symptoms.

Animal studies in a variety of rodent and primate species also indicate that regions of the anterior cingulate and functionally related areas of the striatum and thalamus may be one set of circuits critical for the initiation and maintenance of maternal behavior and normal mother-infant interactions (Fleming et al. 1994; MacLean and Newman 1993; Peredery et al. 1992; Slotnick 1967). For example, some of the most commonly reported persistent behavioral changes following anterior cingulate lesions include impaired maternal behavior without adversely affecting fecundity or litter size (Peredery et al. 1992; Slotnick 1967). These impairments include marked alterations or abolition of nest building, postpartum licking, pup retrieval, and suckling and are associated with increased pup mortality. Selective impairment in the ability to make species-specific separation distress calls has also been documented following lesions of the anterior cingulate (MacLean and Newman 1993).

The Role of Central Oxytocinergic Pathways

A second line of evidence concerns the possible role of central oxytocinergic pathways in both obsessive-compulsive disorder and maternal behavior. Oxytocin is a cyclic nonapeptide with cysteine residues at the 1 and 6 positions which form a disulfide bond. Although some nonmammalian vertebrate species, such as fish and amphibians, have oxytocin-related genes, the exact sequence for oxytocin is uniquely mammalian. It is perhaps no coincidence that oxytocin's best known functions, milk ejection and uterine contraction for viviparity, are two quintessential mammalian traits. The regulatory elements in the 5′-flanking region of the oxytocin gene have not been fully characterized (Grainer and Wray 1992), but they do include a composite hormone response element for members of the steroid superfamily of receptors (Adan et al. 1993; Burbach et al. 1993).

Oxytocin is synthesized primarily in two hypothalamic nuclei—the paraventricular (PVN) and the supraoptic (SON) nuclei, where it is cleaved from a precursor molecule (Brownstein et al. 1980). The traditional description of oxytocin focuses on its synthesis in magnocellular secretory neurons of the PVN and SON that project to the posterior pituitary. More recently, this view has been extended to include a dense network of nonpituitary projections (Buijs 1978; Sofroniew and Weindl 1981; Swanson and Kuypers 1980). Oxytocin can be released directly into the cerebrospinal fluid from dendrites in the walls of the third ventricle (Dogterom et al. 1977; Mens et al. 1983; Perlow et al. 1982). In addition, oxytocin fibers, arising principally from small cells (parvocellular neurons) in the PVN, have been found in many areas of the limbic system (amygdala, bed nucleus of the stria terminalis (BNST), lateral septum, and portions of the hippocampus) as well as in several autonomic centers in the brainstem and spinal cord (Buijs 1978; de Vries and Buijs 1983; Fliers et al. 1986; Sofroniew and Weindl 1981; Swanson and Kuypers 1980; Wagner and

Clemens 1993). Oxytocin may be, in fact, the predominant PVN peptide with autonomic projections to both sympathetic and parasympathetic centers in the brainstem and spinal cord (Sawchencko and Swanson 1982).

Central oxytocin receptors are present in only a few discrete forebrain regions in the rat. The major loci are the posterior border of the anterior olfactory nucleus, the taenia tecta (rostral root of the hippocampus), the lateral segment of the BNST, the dorsal-medial aspects of the caudate, the central nucleus of the amygdala, the ventromedial nucleus of the hypothalamus VMN, and the ventral subiculum. Oxytocin receptors in the forebrain are predominantly in integrative centers rather than primary sensory or motor areas as seen with several other neuropeptide receptors, such as corticotropin releasing factor (De Souza et al. 1984), somatostatin (Reubi et al. 1986), and opioids (Mansour et al. 1987). In addition, oxytocin receptors are found in several brainstem autonomic centers including the dorsal motor nucleus of the vagus and the nucleus of the solitary tract (Tribollet et al. 1990).

Species differences in brain oxytocin binding are notable. The distribution of oxytocin binding in the brains of rats, voles, hamsters, mice, guinea pigs, and humans show marked differences (Dubois-Dauphin et al. 1992; Elands et al. 1988; Freund-Mercer et al. 1987; Insel and Shapiro 1992; Loup et al. 1991; Tribollet et al. 1992). In studies of 12 human brains (aged 40 to 81 years), intense oxytocin binding was observed in the basal nucleus of Meynert, the nucleus of the vertical limb of the diagonal band of Broca, the ventral portion of the lateral septal nucleus and adjacent areas of the BNST, the anterior and posterior areas of the hypothalamus, the pars compacta of the substantia nigra, the substantiae gelatinosa of the caudal trigeminal nucleus, and the dorsal horn of the upper spinal cord (Loup et al. 1989, 1991). Less intense and variable oxytocin binding was observed in the globus pallidus and its subcommissural extension, the ventral pallidum (Loup et al. 1991). Although not observed in animal studies, the binding in the pars compacta of the substantia nigra (where dopaminergic projections to the caudate, putamen, and globus pallidus originate) was seen in all of the brains examined. No sex differences were observed.

Although the distribution of receptors is identical in male and female brains (Tribollet et al. 1990; although also see Bale and Dorsa 1995), oxytocin receptor distribution in the infant is markedly different from the pattern seen in adults. Binding to cingulate cortex, globus pallidus, and midline nuclei of the thalamus is intense only in the infant, while certain areas with high levels of binding in the adult brain, BNST and VMN, show virtually no receptors prior to sexual maturity (Shapiro and Insel 1989; Snijdewint et al. 1989; Tribollet et al. 1989). The significance of this transient expression of oxytocin receptors in development is not understood, but similar patterns of evanescent receptors during ontogeny have been previously reported for several other neuropeptides (Insel et al. 1988; Palacios et al. 1988; Quirion and Dam 1988).

Given this background, we now consider the evidence linking central oxytocin pathways with the initiation of maternal behavior and with the pathobiology of obsessive-compulsive disorder. The past decade has seen the emergence of a substantial literature indicating that oxytocin is centrally involved in a broad range of behaviors, including the initiation of parental behaviors and infant attachment (Insel 1992*a*, 1993). Specifically, several (Fahrbach et al. 1984*a*, *b*; Pedersen et al. 1982; Pedersen and Prange 1979; Wamboldt and Insel 1987), but not all (Bolwerk and Swanson 1984; Rubin et al. 1983) studies have reported that oxytocin given centrally (but not peripherally) to virgin female rats induces full maternal behavior within minutes.

It is important to realize that virgin female rats display little interest in infants and when presented with foster young will either avoid or cannibalize them (Rosenblatt and Siegel 1981). Just prior to parturition (or following specific steroid regimens to mimic the physiologic changes of parturition), there is a rapid, dramatic shift in motivation from a lack of interest to a driven, relentless pursuit of nest-building, retrieval, licking, grouping, and protection of pups (Numan 1988). No other peptide or drug has been shown to induce maternal behavior so quickly in virgin females.

However, oxytocin does not work alone. In all the studies demonstrating an induction of maternal behavior following central oxytocin administration, the response was dependent on priming with gonadal steroids. No effects of oxytocin are observed in ovariectomized females unless they are treated with estradiol. The sites at which oxytocin might function to induce maternal behavior remain incompletely defined, although results from site-specific injections implicate the medial preoptic area and the ventral tegmental area (Pedersen et al. 1994).

Does oxytocin have a physiologic role in the induction of maternal behavior? This question can be answered by blocking central oxytocin pathways using centrally administered antagonists, antisera, or lesions. Studies with all of these methods demonstrate that following either experimentally simulated (Pedersen et al. 1985) or natural parturition (Insel and Harbaugh 1989; van Leengoed et al. 1987), the onset of maternal behavior can be blocked by oxytocin antagonism. One key feature of these studies is that oxytocin antagonists do not appear to disrupt maternal behavior per se, they block its initiation. The same intervention following parturition when maternal behavior is established is without effects. It should also be noted that interventricular injections of oxytocin suppress infanticide in mice (McCarthy et al. 1986; van Leengoed et al. 1987).

It appears from these studies in rodents that oxytocin's effects within the central nervous system influence the initiation of maternal behavior consistent with this peptide's role in peripheral tissues for the induction of labor and milk ejection. Further evidence for this apparent effect of oxytocin on maternal "motivation" comes from studies in virgin sheep, which resemble nulliparous rats in their normal absence of maternal interest. Vaginal-cervical stimulation, a potent stimulus for both central and peripheral oxytocin release (Kendrick et al. 1988), induces the rapid onset of maternal behavior in the steroid-primed ewe (Keverne et al. 1983). More important, central but not peripheral oxytocin administration increases maternal interest in nulliparous ewes, shifting their behavior toward newborns from avoidance or aggression to exploration and caretaking (Kendrick et al. 1987).

In an effort to examine the role of oxytocin in obsessive-compulsive disorder, we compared cerebrospinal fluid levels of oxytocin from a total of 116 individuals—39 patients with obsessive-compulsive disorder, 33 patients with Tourette's syndrome, including 14 with obsessive-compulsive disorder as well as Tourette's syndrome, and 44 normal controls (Leckman et al. 1994*a*, *b*). We collected lumbar cerebrospinal fluid at midday in a standardized fashion. We also collected family study data on each of the subjects in order to determine which subjects had a positive family history for Tourette's syndrome, obsessive-compulsive disorder, and related syndromes.

We found marked increases in cerebrospinal fluid levels of oxytocin in obsessive-compulsive disorder patients without a personal or family history of tic disorders (figure 2). A possible role for oxytocin in the neurobiology of an independently defined subtype of obsessive-compulsive disorder (patients without a personal or family history of a tic disorder) was further suggested by a correlation between cerebrospinal fluid levels of oxytocin and concurrent clinician ratings of obsessive-compulsive symptom severity ($N=19$, $r=0.47$, $p<.05$). Phenotypically, 75 percent of these subjects had washing and cleaning compulsions as part of their symptom picture.

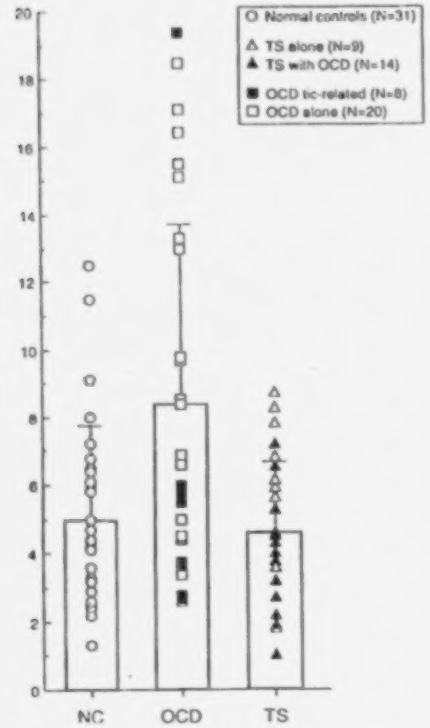

Figure 2 Cerebrospinal fluid (CSF) oxytocin levels in patients with obsessive-compulsive disorder, patients with Tourette's syndrome, and normal control subjects (Leckman et al. 1994*a*). The obsessive-compulsive disorder patients had significantly higher CSF levels of oxytocin ($p<.01$) than either of the comparison groups.

A Hypothesis Concerning the Neurobiological Substrates of Some Forms of Obsessive-Compulsive Disorder

Although these findings have yet to be confirmed by other investigators and so should be viewed with caution, we have hypothesized that some forms of obsessive-compulsive disorder are associated with altered patterns of oxytocin receptor distribution in the brain and that these patterns to some extent resemble those that might be seen in the brains of parents who are deeply under the spell of their new daughter or son. In order to be consistent with the available in vivo neuroimaging data, we hypothesize that oxytocin receptors in the anterior cingulate cortex and the ventral caudate/putamen are upregulated (similar to the distribution seen in rat pups before weaning).

In this model, a lack of oxytocin relative to the number of oxytocin receptors would be anxiogenic. This form of oxytocin-related obsessive-compulsive disorder would be associated with a later age of onset (given the need for gonadal steroid priming) and involve prominent themes of separation anxiety, contamination worries, and a heightened sense of responsibility for maintaining a safe and secure home environment. The elevated levels of cerebrospinal fluid oxytocin seen in some of these individuals may be associated with some abnormality in the number or responsiveness of oxytocin receptors in areas such as the anterior cingulate so that despite high levels of oxytocin synthesis and release, the patients would remain anxious and distressed.

This hypothesis depends in part on the functional interrelationship between the PVN and areas in the anterior cingulate. As early as 1961, it was shown that electrical stimulation of the rostral portion of area 24 of the anterior evokes oxytocin release (Beyer et al. 1961). More recently, investigators have noted higher numbers of cells showing Fos-like immunoreactivity in the cingulate cortex of hormonally primed parturient rat dams immediately following their first behavioral interactions with their newborn pups (Fleming et al. 1994).

This hypothesis is partially an extension of the anxiety-reduction theory of obsessive-compulsive disorder (Miller 1969, 1982; Mowrer 1960). The essence of this theory is that the anxiety or discomfort elicited by obsessive thoughts motivates performance of compulsions and that these acts are reinforced by a reduction in the discomfort that they produce. This theory is also supported by a body of empirical evidence showing that the subjective distress generated by exposure to a symptom-provoking situation is diminished with the performance of compulsions (Rachman et al. 1976; Rachman and Hodgson 1980; Röper and Rachman 1976; Röper et al. 1973).

From a physiological perspective, this theory is consistent with oxytocin's role in maintaining and reestablishing homeostasis in concert with multiple other neuroendocrine and neuronal systems (Richard et al. 1991; Swanson and Swachenko 1980). It is also quite likely that central oxytocinergic pathways are just one system among many that contribute to the modulation of evolutionarily conserved patterns of parental behavior and associated mental states (including the possible vulnerability to some forms of obsessive-compulsive disorder).

Further Prospects for a Darwinian Reformulation of Psychiatric Nosology

If certain adaptive sets of human mental states and behaviors, including early parental love, are evolutionarily conserved, and if this conservation is reflected in the functional neurobiology of the developing organism, then it may be reasonable to conclude that these conserved systems are also intimately involved in our species' vulnerability to certain forms of psychopathology. As we have discussed elsewhere, several candidates come to mind. Autism and related conditions may occur when there has been a failure of one or more of these conserved, attachment-related neurobiological and behavioral systems to develop normally (Panksepp 1992). Dysregulation of these systems may also lead to a broad range of dangerous and injurious behaviors that extend from self-injurious or suicidal behavior to acts of violence directed toward infants or estranged sexual partners (Marzuk et al 1992; Resnick 1970). It is even possible that these highly conserved systems can be co-opted by drugs of abuse (Sarnyai and Kovacs 1993). Although a full exploration of these disorders is beyond the scope of this review, it may make sense in closing to outline some of the issues that will need to be addressed in taking a Darwinian point of view.

First, in considering any psychopathological condition, it may be reasonable to ask what conserved set of adaptive behaviors failed to develop, is broken, or is dysregulated. The identification of these adaptive systems and their neurobiological substrates should provide a guide for both clinical studies and more basic genetic and neurobiological studies. Clinical approaches require identifying the relevant dimensions to measure, while genetic and neurobiological studies focus on which genes and developing brain circuits are involved in generating and mediating these behavioral dimensions.

Second, it may be fair to inquire if there are any advantages to displaying features of these mental disorders, like malarial resistance among individuals with sickle cell trait, so that having a low genetic "dose" of vulnerability confers advantage while higher doses diminish fitness? A corollary is that such advantages or disadvantages are likely to be situation specific. For example, some traits viewed as maladaptive today may simply be the result of a "bad fit" with our rapidly changing, information-intensive mass culture. For example, the diagnostic criteria for what is currently termed attention-deficit hyperactivity disorder (American Psychiatric Association 1994) focuses on potential disruptive behaviors observed in the classroom. Many of these same children show few behavioral problems in less distracting environments. One can also imagine threatening environments where distractibility and attention to novel stimuli would confer selective advantages. The answers to these questions may be useful in developing interventions that focus on optimizing the individual's environment for purposes of rehabilitation and refuge as well as improved long-term adaptation.

Whether the application of Darwinian principles will alter the study of psychopathology remains an open question. However, there is reason to be hopeful that it will provide a fruitful perspective in considering these phenomena. In this regard, we are encouraged by the pioneering work of other investigators who have adopted this same point of view (Crow 1995; Klein 1993; McGuire et al. 1992).

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Commentary

Developmental Plasticity and Continuity in Social Interactions: Attachment and Aggression

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Reciprocity and bidirectionality are nuclear concepts in modern accounts of social development (e.g., Patterson 1982; Sameroff and Suomi 1996). Individuals influence, and are influenced by, the actions of others. These interchanges are assumed to provide the substrate for future actions. While the developmental model readily accounts for adaptation and plasticity, it encounters problems in explaining why some social patterns are predictable over the long term. Specifically, *if plasticity and change are inevitable in social development, how can continuity and stability be achieved*? Further, given the inevitability of changes in basic emotional, morphological, and neurobiological processes, how is continuity in social behavior possible?

There is no question but that social continuity occurs. For example, longitudinal studies in humans show that aggressive patterns at maturity can be predicted with reasonable accuracy, given the rate of hostile interactions observed in middle childhood (e.g., Magnusson 1988; Pulkkinen 1982; Cairns and Cairns 1994). Moreover, longitudinal studies of aggressive behaviors in animals have described some of the correlated processes that give rise to continuity and change. However, data from longitudinal studies of both humans and animals suggest a couple of twists in the developmental paradox, namely:

- There is greater conservatism and continuity in behavior over development than might be anticipated from the concepts of reciprocity and bidirectionality.
- There is greater plasticity and change over development than might be

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> anticipated if there were early canalization, whether by genes, embryogenesis, or early experience.

The remainder of this chapter outlines some thoughts on the developmental dilemma, with special focus on the phenomena of attachment and aggression.

Plasticity and Continuity in Social Attachment

By the mid-1960s, sufficient empirical data had been gathered on the effects of childrearing to question the utility of the then-dominant model (i.e., social learning theory). The propositions of social learning on the effects of parenting upon child behavior disorders were only modestly supported in studies of families (Bandura 1960; Sears et al. 1957; Yarrow et al. 1968). Attempts to replicate the few significant findings tended to be unsuccessful. This state of affairs prompted Yarrow and her colleagues to make the courageous observation that:

> Childrearing research is a curious combination of loose methodology that is tightly interwoven with provocative hypotheses of developmental processes and relationships. The compelling legend of maternal influences on child behavior that has evolved does not have its roots in solid data, and its precise verification remains in many respects a subject for future research. (Yarrow et al. 1968, p. 152)

To be sure, the evidence was not entirely negative. Social learning theory had been tolerably effective in accounting for the results of short-term experiments in children, such as the effects of television in stimulating novel behaviors. But it did not fare well in its primary task of accounting for the long-term effects of variations in parenting practices.

Developmental models of early experience and social attachment emerged as attractive complements to the nondevelopmental assumptions of social learning theory. Comparative work with primates and other nonhuman mammals provided fascinating findings on early experience effects. The work of Scott (1945), Harlow (1958), Kuo (1967), Schneirla (1966), Jensen (1967), Hebb (1953), Hinde (1966), and Bateson (1966), among others, set the stage for a major shift in models and methods.

In a series of comparative studies, we found that attachment phenomena were highly malleable early in the young organism's life and remained sensitive to modification over development (e.g., Cairns 1966*a*, b, 1996; Cairns and Werboff 1967). These findings from studies of young lambs and dogs were later replicated in nonhuman primates by Mason and Kinney (1974), Novak and Harlow (1975), and Suomi and Harlow (1972). Beyond the plasticity of social attachment, we found that the social changes occurred very rapidly. Attachment modification was typically initiated within 2-8 hours in these species and consolidated within 1-2 weeks (Cairns 1979).

Such rapidity in the reversal of attachment seemed to make good sense from a developmental and evolutionary perspective. The survival of most mammalian young presupposes rapid accommodation and the formation of a new attachment if the old one is broken. If the mother rejects the offspring, dies, or becomes otherwise removed, a lengthy delay in the infant's accommodation to a new surrogate could be catastrophic. Accordingly, we found that a fine balance existed between the processes of conservation and change. Experimental analyses indicate that stability mechanisms give way to change mechanisms within hours and days rather than weeks and years (Cairns 1996). A parallel balance of timing seems to hold for human infants (Fleener 1973; Cairns 1996). Fleener (1968, 1973) demonstrated a powerful induction of social attachment in 7- to 14-month-old babies after only 3.5 hours of interaction.

In brief, shifts in the quality, form, and intensity of the infantile attachment seem inevitable. What does endure in the young over time are the effects of habituation (i.e., early exposure reduces neophobia at maturity). Moreover, species-typical social and sexual preferences are conserved over development and over generations, even when young mammals are raised with alien species (Kuo 1967; Cairns 1979). Although the mechanisms that account for these forms of developmental and intergenerational conservation have yet to be fully specified, self-stimulation across development is doubtless involved (Dmitrieva and Gottlieb 1992). So are precisely tuned species-typical sensory and receptor capabilities in mothers and offspring (Hofer, this volume).

It seems reasonable to conclude that behavioral, endocrine, and neurobiological processes provide a network of constraints that promote the physical survival of the young organism and, in the long term, intergenerational transmission. As immature processes become superfluous and are eliminated, fresh developmentally appropriate processes come into play.

One of the reasons for continued controversy in the attachment literature has been the failure to distinguish between attachment phenomena in infants and attachment phenomena in mothers and fathers. Not only do the maternal and infant effects seem to be mediated by different learning and neurobiological processes, they occur in different timeframes. Maternal attachment, for example, clearly occurs in the immediate postnatal period. However, the onset of social attachment in most infant mammals (including ungulates, canines, and primates) occurs only after the maturation of visual, chemical, and auditory sensory systems that permit distal perception. Failure to distinguish between parental and infantile attachment processes, and how they may be linked together, has been a common but unfortunate oversight.

More broadly, it appears that continuities in the specific social attachment of infants do not reflect fixed structures in behavior or biology so much as they represent a tension between continuity and change mechanisms. What is commonly viewed as stable and constant is more appropriately conceptualized as dynamic equilibrium. Social interactions are more akin to fluid metabolic processes than they are to sculpted physiological structures. The processes that account for both stability and change are open to investigation and manipulation over ontogeny.

Plasticity and Continuity in Aggressive Interactions

Beyond obvious functional differences in attachment and aggression, there are some less obvious differences in developmental timing. For example, social attachment patterns are assumed to be organized and activated in infancy. By contrast, aggressive patterns are ordinarily assumed to be organized and activated in childhood and adolescence. These timing differences seem to have had an impact on whether attachment is somehow presumed to be more basic than the other.

Perhaps this is why so few developmental studies of aggression have been conducted in animals.* Because of our special concern with plasticity and change, we focused on the enduring effects of early experience. Is there a sensitive period for early stress or other manipulations that are purported to be related to aggression? In the process of attempting to answer that question, we were catapulted into studies of the plasticity of both genetic and early experience effects on aggressive patterns. For this work, mice were selected, in part because the primary work on behavioral genetics has been conducted with mice, and in part because their aggressive patterns have been extensively investigated (e.g., Brain et al. 1989; Green 1978). Relatively little work had been completed, however, on the development of aggressive patterns in mice.

Once the key parameters of the development of the phenomenon were outlined empirically, rapid progress became possible (Cairns 1973). Selectively breeding for genetic lines of mice that show differences in aggression proved to be an economical project. Lines of males that show virtually no overlap in attacks and fighting can be produced through selective breeding in the surprisingly short time of 34 generations (see Lagerspetz and Lagerspetz 1971; Cairns 1976; Cairns et al. 1983; van Oortmerssen and Bakker 1981).

In the developmental studies, we found powerful effects of early isolation and handling experience on aggressive behavior in mice. We also learned that the direction of these effects on aggression—whether they produced an increase or decrease in aggression—depended upon the biology of the animal and the timing of the experience. The same objective experiences produced opposite effects. The outcome seemed to fit Allport's (1937) metaphor on differential effects of early experience: "The heat that melts the butter hardens the egg."

Specifically, we found no systematic differences in aggressive behavior as a function of genetic line if the animals had been reared in small groups of conspecific males. But huge line differences were observed among their brothers who were reared in isolation. That is, if males from each line are placed in solitary confinement from weaning to maturity, then tested, the two lines differ dramatically in the likelihood of attacking unfamiliar male mice. High line animals become extremely aggressive, while low line animals rarely attack (Cairns 1996). Recently, Gariépy and his colleagues extended these results by showing that the effects were reversed when the animals were handled rather than isolated. Males in the high-aggressive lines responded to handling by fewer attacks at maturity, while males in the low-aggressive lines responded to handling by more attacks.

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*There are some important exceptions, including the work of Brain (e.g., Brain et al. 1989), Scott (1958, 1966), and Kuo (1967).

Similar paradoxical effects are observed in the females of the two aggressive lines. In female mice, the gender-appropriate condition for eliciting aggression is to test them during postpartum, 3-18 days following parturition (Green 1978). Accordingly, it is primarily when females are assessed during this period that differential effects are observed between the lines. When an unfamiliar male or female mouse is placed in the litter compartment during this period, maternal females from the high-aggressive line immediately and viciously attack the intruder. In contrast, postpartum females in low-aggressive lines rarely attack (Hood and Cairns 1989). The upshot is that line differences among females in aggressive behavior during the postpartum period equal or exceed line differences among males after isolation. Otherwise, females from the two lines show few differences in aggressive behavior.

So much for early plasticity. Is there stability in the behavior and what may account for continuity of aggressive behavior over time in these species? In a review of this matter, we found genetic line and individual-difference predictability over development (Cairns and Hood 1983). What factors promote continuity in the midst of change? We anticipated neurobiological constraints in the two lines might contribute to continuity over time. The results support that proposition. Two genetic lines have shown consistent differences in dopaminergic activity (D1, D2) and associated receptor sites in the nucleus accumbens and caudate nucleus (Gariépy et al. 1996). These line differences in neurobiology are linked, in turn, to line differences in behavioral reactivity to social stimulation. In dyadic tests, this neurobiological linkage to reactivity is associated with attack instigation and attack persistence. But it should also be underscored that the experiences of animals trigger changes in hormones and neurobiology. Specifically, defeat reduces levels of testosterone regardless of genetic line, and group rearing modifies line differences in dopamine concentrations (Gariépy et al. 1996).*

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*Much of the recent interest in the neurobiology of aggression was inspired by work begun in the late 1970s in Stockholm. Åsberg, Shalling, and their collaborators discovered a linkage between neurobiological status in young male adults and impulsive suicide (e.g., Åsberg et al. 1987). Further work by these investigators and Virkkunen in Helsinki and Linnoila and Brown in the United States confirmed a linkage between neurobiology and impulsive aggression, with a special focus on serotonergic transmission (e.g., Linnoila et al. 1983). Moreover, there is a link between these neurobiological dispositions and specific genetic loci, as demonstrated in Goldman's molecular genetics laboratory (Stoff and Cairns 1996).

By way of summary, developmental studies of aggression in selectively bred lines of mice suggest the following generalizations:*

- Social interactions, including aggressive behaviors, play a key role in fine-tuning the biological organization of individuals and in the structuring of their physical and social environments. Social interactions thereby serve distinctive and unique functions in organismic and contextual adaptation; namely, (a) they provide for rapid and/or reversible accommodations, and (b) social interactions occur in the space between organisms and their environments, and they affect both.
- Single variables rarely act alone or in isolation from the context. Typically, alignment arises in development between neurobiology, social contexts, and interactional behaviors as a consequence of the bidirectional and reciprocal relationships. The resultant "package of variables" provides for stability and predictability.
- Conservation in aggressive behavior is therefore supported by the correlated constraints from within and from without the individual. The upshot is that behavior organization tends to be continuous and conservative over time despite the fluidity and change inevitable in development.
- Behavior most modifiable by variation in experience may also be particularly sensitive to genetic variation (Fuller 1967). Although most properties of social systems are closed to rapid ontogenetic and microevolutionary change, some key elements remain open to change through variations in developmental timing (de Beer 1958). Heterochronies can provide the first stage for cross-generational changes in trajectories of aggressive behaviors.
- Accordingly, social behaviors, including aggressive interactions, may constitute a leading edge of biological change. Once interactions prove effective, they create the scaffold for further changes in neurobiology, morphology, and physiology in ontogeny. They also can create conditions that influence genetic selection and transmission across generations (Bateson 1991; Cairns et al. 1990). The upshot is that aggression and other social actions are among the first features to be influenced by selection pressures, not the last.

Related Contributions

Four companion chapters in this volume address aggressive behavior, early experience, genetic influence, and the psychobiological roots of attachment. Because of their immediate relevance to the issues covered in this essay, it seems appropriate to offer some comments on their contributions.

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* The empirical evidence for these generalizations is summarized elsewhere (Cairns 1996; Cairns et al. 1990).

Coyle draws attention to the environmental constraints that figure importantly in any account of social behavior stability and change. Accordingly, we need to have a better understanding of the social ecology and the stability of "toxic environments" in order to understand behavioral pathologies and how to change them. More broadly, the problems of individuals and societies appear in correlated packages, and the problems of violence, school dropout, and teenage parenthood should not be divorced from the social contexts in which they occur. Configurations of individual problems are correlated with toxic environments. Hence, there is a fail-safe quality about certain social outcomes—for good or for ill—where individuals are embedded in a network of relationships. The support for social behavior stability—whether adaptive or deviant—can come from without as well as within.

In a closely related chapter, Hofer focuses upon the organizing influence of evolutionary history of individuals in constraining the kinds of interactions that are observed. For example, certain maternal and attachment behaviors do not have to be reinvented anew each generation. Genetic background and evolutionary history provide safeguards to protect the integrity of the maternal-infant interaction. In his elegant work, Hofer has plotted the marvelous synchrony between biology and behavior of both mothers and infants critical for attachment formation. The afferent stimulation of the young—including infantile vocalizations—stimulate caretaking, oxytocin release, and lactation, creating the conditions for the mutual support of the biology and behavior of both the mother and the infant. In the perinatal period, the maternal condition of the female is established partly by interactions with the young.

But just as infants and mothers have evolutionary histories, they have the shaping influence of developmental changes and interactional experiences. As young grow up, and as their behaviors and biologies change, so do the behaviors and biological states of the mother, renewing the cycle necessary for reproduction. On this score, plasticity in social behavior is not limited to infancy: it continues through maturity, and it is jointly determined by inevitable internal changes in the individual and changes in the others with which the individual interacts.

Emde addresses the problem of how to integrate a concern with developmental dynamics and issues of human behavior genetics. As he observes, genetic designs can be invaluable in helping understand the operation of social-contextual influences, and vice-versa. Emde's recent work has demonstrated this proposition in the study of children in the context of families. Indeed, it is when we control the rest of the sources of variance in developmental rearing and assessment that genetic effects appear to be overwhelming. It also points to the relativity of any statement of how much, particularly because of the usual confounding of differential interactions as infants become children, adolescents, and adults.

Leckman examines the evolutionary, developmental, and neurobiological substrates of love, and how this helps us understand behavioral conservation. The theme is entirely compatible with the preceding chapters, though the specific hypotheses are different. This reminds me of the early attachment work by Klopfer with goats (Klopfer et al. 1964), where oxytocin release within the first 5 minutes of birth was associated with maternal acceptance and recognition. Interestingly, the same magic does not work with the lambs, in that they do not form the powerful selective bond for 2–5 weeks. This difference possibly reflects the asymmetry between the mother's attachment to her baby and the baby's attachment to the mother. There seems to be clearly an emotional counterpart to romantic love that extends beyond sexual arousal. And it provides support for the continuation of the relationship.

But separation happens, divorces occur, and children get neglected. Is the neurohormonal support a determinant, an outcome, or part of the correlated complex that supports the relationship? This speaks to the questions that we raised earlier—and brings up a new one. What are the functions of close relationships in organizing both internal states and external contexts?

Concluding Comments

One of the challenges of modern biobehavioral integration has been to solve the paradox of how internal and external adaptive systems are linked together. How can optimal levels of behavioral plasticity and novelty be maintained if physiological and neurobiological systems are biased toward conservatism and continuity? This question speaks to how organisms can serve two masters at the same time—the internal forces within that promote intraorganismic balance and synchrony, and the external forces without that demand changes in environments and interactional patterns.

Developmental considerations suggest that there is special folly in limiting one's search for a specific gene—or specific neurotransmitter—that controls attachment or aggression. Genetic and neurobiological contributions become coalesced into configurations that influence social interactions; they rarely function as isolated variables. Social interactions themselves are plastic and dynamic over time and space, and so, apparently, are the neurobiological events that become correlated and aligned with them.

Yet an appreciation of behavioral and developmental dynamics does not preclude precise analyses of proximal processes. To the contrary, it can help organize the research agenda. Accordingly, an immediate goal for future research is to determine how serotonergic and dopaminergic systems are linked together and how they are linked with aggressive patterns over time. Similarly, oxytocin and the family of hormones at postpartum have been associated with the onset of maternal attachment. It is now a reachable goal to learn whether developmental change in this hormonal substrate is linked to change in maternal attachment. It is also feasible to determine whether parallels exist in the attachment patterns of infants. Recognition of the complexity of development is the first step toward understanding its coherence and simplicity.

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Developmental Plasticity—Integration

Integrating Across Levels of Complexity

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ABSTRACT

Progress in the understanding of human behavior has inevitably led to increasing specialization in the behavioral sciences and decreasing contact between disciplines. Yet full understanding of practically every type of behavior, and especially of its development, requires us to draw on diverse sources of data concerned with different analytical levels.

The study of behavior can be conceived as involving a number of levels of complexity, including a number of levels of intraorganismic physiological and psychological processes, individual behavior, interactions between individuals, relationships, groups, and societies. Behavior at successive levels involves additional properties and requires additional explanatory concepts. Each of these levels affects and is affected by others, and also affects and is affected by the sociocultural structure of norms, values, beliefs, and institutions in the society. Each level, including that of the individual, is thus to be seen not as an entity but as a process in continuous creation, maintenance, or degradation through the agency of the dialectical relations between levels.

To unravel this complexity, a point of entry is necessary. The use and the limitations of the concept of "relatively stable characteristics" is discussed. These are characteristics that are more or less similar between individuals, so that the environmental features necessary for their development must be present for all individuals. This approach is illustrated by the development of snake phobias, the development of gender differences, and the incidence of international war.

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At the beginning of this meeting, Dr. Kupfer emphasized the importance of integrating the molecular, cellular, systems, and what he called the "behavioral" level. The head of OBSSR later included in one box "behavioral, social, and environmental factors." In this chapter, I emphasize the importance of dissecting this box. In doing so, I shall put Dr. Kupfer's three levels into one box of "physiological" or "psychological" processes, though with full respect for the complexity of what is embraced in that box, and focus on "higher" levels of integration.

Development depends on reciprocal processes between embryo, fetus, baby, child, adult, and the environment. That environment not only impinges on, but also is created by, the individual at each stage in development. Individuals respond selectively to the environment, assign meanings to it, change it, and are changed by it. So far as psychological development is concerned, the most important part of that environment is constituted by other persons. Individuals interact with others, form relationships with them, and participate in groups. They are affected by, and help to maintain and create, the sociocultural structure of beliefs, norms, values, and institutions with their constituent roles. All of these are distinct but interrelated issues and demand our attention. The rub is that these aspects of behavior have become the subject matter of different disciplines—physiology, individual psychology, social psychology, sociology, and anthropology, among others. And new subdisciplines are always being born—"behavioral neurogenetics" was a new one for me at this meeting.

I would wish to argue that full understanding of the development and causation of any aspect of behavior demands that we cross and recross between successive levels of complexity. As an initial example, consider the integration of nest-building behavior in canaries.

Interactions Between Physical Environment, Behavior, and Endocrine State

When a female bird builds a nest, it has to be ready just in time for the eggs to be laid. How is this achieved? A number of years ago we analyzed the nest-building of female canaries from this point of view. The data demonstrated a continuing series of interactions between changes in the physical environment, the female's behavior, and her endocrine state. These can be summarized briefly as follows.

Environmental factors, acting via the hypothalamic-pituitary-gonad system, result in gonad development and oestrogen release. As a result, the female becomes responsive to the male's courtship. The formation of a relationship with the male results in further development of the female's reproductive system, and the changes in her hormonal state induce nest-building, though the effectiveness of those changes is influenced also directly by external factors, such as daylength and exposure to male song.

Nest-building results in the construction of a nest, and the female is affected by stimuli from the nest that she has built acting through the skin of her ventral areas. Further endocrine changes enhance the sensitivity of this area by the development of a brood patch, involving loss of feathers, increased vascularity, and heightened tactile sensitivity. Stimuli from the nest result in a change in the nature of material incorporated into the nest from grass to feathers, a decrease in nest-building behavior, and further reproductive development.

Thus, the reproductive cycle involves a continuous interplay between environmental factors, hormonal factors, and behavior. The behavior cannot be fully investigated in the absence of some physiological analysis nor can the physiology be investigated properly in the absence of some behavioral understanding (Hinde and Steel 1966, 1978). This is old work now, and has been overtaken by the research on the control of reproduction in the ring dove by the late Daniel Lehrman and extended by Cheng and colleagues at Rutgers University, but it demonstrates how the female canary's behavior changes her environment, and the new stimuli received as a consequence produce further endocrine and behavioral changes. In the same way, the human infant creates new environments as the milestones of standing, walking, and talking are achieved.

More importantly, this work illustrates the need to cross between levels of complexity even in the relatively simple case of avian reproduction.

Additional Complexities in the Human Case

In analyzing human behavior, it is convenient to distinguish a number of interrelated levels of complexity—intraindividual processes which may be described in psychological or physiological terms, individual behavior, short-term interactions with others, relationships (consisting, in behavioral terms, of series of interactions between two individuals who know each other, such that each interaction is influenced by preceding ones), groups, societies, and the sociocultural structure (figure 1). Three points about these levels may be emphasized:

1. Each involves properties that are simply not relevant to the level below. For example, the relationships in a group may be arranged hierarchically, linearly, or in many other ways; these issues are not relevant to the properties of a relationship.

2. Different explanatory concepts can usefully be invoked at each level. For instance, at the interactional level, we might ascribe a particular conflict between two siblings to competition over a toy; but at the relationship level, we might explain the frequency of their conflicts in terms of sibling rivalry.

3. Each of these levels affects, and is affected by, others. Thus, the course

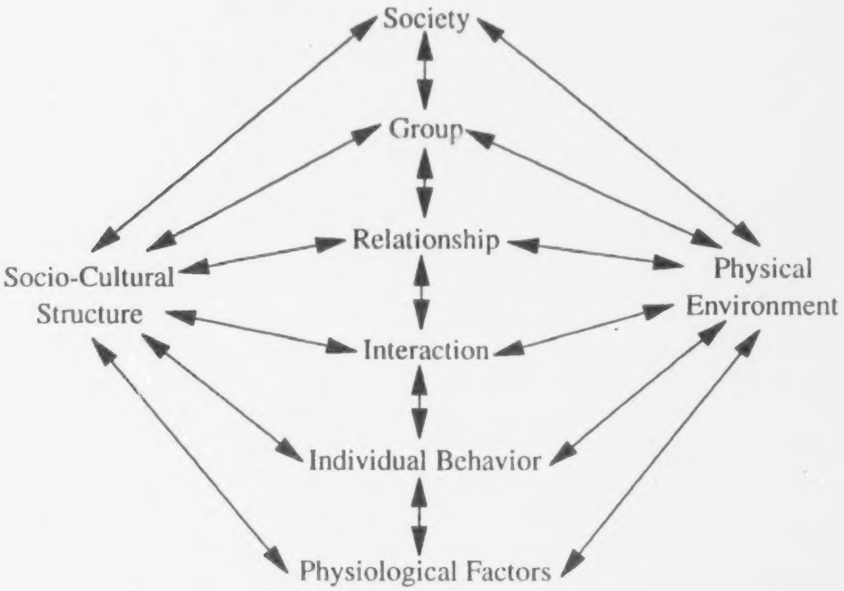

Figure 1. The dialectical relations between levels of social complexity.

> of an interaction is affected by the individual propensities of the participants; the nature of a relationship depends on the properties of the component interactions; the properties of the interactions are influenced by the relationship in which they are embedded; and the quality of the relationship and its interactions affect the behavioral propensities of the individual. Furthermore, the properties of each level affect and are affected by the sociocultural structure. For example, the more couples live together before marriage, the more acceptable such behavior becomes, and the more acceptable it becomes, the more prone are couples to behave in that way. At the individual level, these influences involve behavioral, cognitive and affective processes, mediated (once a certain stage of development is passed) by the meanings attributed to events and situations. Thus each level, including that of the individual, is to be seen not as an entity but as involving processes of continuous creation, change, or degradation through the dialectical relations within and between levels (Hinde 1991*a*, 1997).

In this context, it is salutary to reflect on the illusion that we have a constant self throughout life. Not only do we change with age, but a moment's introspection convinces us that the nature of our affective experience, cognitive processes, and behavior changes according to the situation we are in (cf. Goffman 1963). Perhaps this has implications for the nature of brain functioning which deserve further consideration.

An Illustration

As an illustration, consider the responses of humans to snakes. Toddlers, who have been brought up in an institution and have never previously seen a snake, pay close attention to a snake, and may show fear, when 1-2 years old. The nature and intensity of their response varies with that of their caregiver, who in normal circumstances would probably be a parent (Prechtl 1950). If the caregiver responds with fear, the child is likely to acquire a long-term fear of snakes by a process known as "social referencing."

A comparable situation occurs in rhesus monkeys. Experimental data show that wild-reared, but not laboratory-reared, rhesus show fear of snakes, and that laboratory-reared monkeys shown a videotape of a wild-reared monkey showing fear of a snake acquire a fear of snakes, but a similar tape showing a wild-reared monkey displaying fear of a flower does not have such an effect (Mineka 1987). The evidence thus indicates that snakes are salient for both rhesus monkeys and humans, and that both have a propensity to learn to fear snakes which is evoked by social referencing.

The importance of such basic predispositions is illustrated more generally by the fact that all the true human phobias—of falling, darkness, and so on—concern issues that would have been of danger to our remote ancestors under the conditions in which our early human ancestors lived. Cars and bombs and other modern dangers are not the subject of phobic fears (Marks 1987).

However, in humans another issue enters in—the way in which snakes are portrayed in mythology. In the west, they are symbols of evil, as portrayed vividly in the Rubens paintings of the lost souls going down into Hell with snakes gnawing at their genitals. In other cultures, the salience and power of snakes is interpreted in other ways. In southern India, they are generally regarded with aversion, but they are also associated with the deities and seen as a source of universal energy and cohesion. In summary, then, to understand responsiveness to snakes, one must come to terms with dialectical processes between basic individual predispositions, relationships with caregivers, and the sociocultural structure.

Breaking Into the Dialectics

Fear of snakes is a relatively simple, though paradigmatic, case. For many aspects of human behavior, it would clearly be impossible to disentangle all the dialectical issues implied by figure 1. But progress can be facilitated if one can identify a point of entry into the system. A few decades ago, a useful approach might have been to use "human universals" as a starting point, but the search for human universals has had a long and not very respectable history (Count 1973; Geertz 1973). Nor are attempts to identify "innate" or "instinctive" aspects of behavior of any use (Oyama 1985). However, it is useful to conceive of characteristics as arranged along a continuum from those that are relatively stable to environmental influences (other than those acting ubiquitously on the group of individuals considered) to those that are relatively labile. Thus, some characteristics of structure and behavior appear in virtually the whole range of environments in which life is possible, others always appear if the conditions are within certain limits, and others are closely dependant on environmental circumstances. Of course, all characteristics have some variability: we all have noses, but no two noses are identical. But it is possible, as an heuristic device, to identify "relatively stable characteristics" (RSCs)—the relative stability assuming certain limits of environmental conditions (e.g., Greenfield and Childs 1991). For present purposes, I shall use it for characteristics that are relatively stable for all humans, or for all humans of an age/sex class.

Such RSCs may involve aspects of perception (e.g., distinguishing figure from ground), motor patterns (smiling, sucking), responsiveness to stimuli (maternal responses to her infant's cries), certain aspects of motivation (to eat, to form relationships), certain cognitive processes (classification, construction of a self-system), predispositions to learn, and so on (Hinde 1991*a*).

Any one RSC may have developmental consequences for many aspects of behavior. For instance, humans have a need to feel that they can cope or control their environment—though coping may involve opting out of attempts to control. This need contributes in turn to the need for autonomy within personal relationships (e.g., Baxter 1990), to many other aspects of behavior within relationships (e.g., Hinde 1997), to seeking for status, and so on.

It must be emphasized that the RSC is only an heuristic device. What seem to be among the more important RSCs are often somewhat intangible, such as propensities to learn this rather than that. Others may not be as simple as they seem at first at sight. For instance, we have propensities to learn and use a spoken language, and this has many consequences for our cognitive capacities, ability to form relationships, social behavior, sociocultural structure, and so on. But language depends on a variety of ancillary mechanisms—adaptations to the sensory and motor systems as well as (probably) special language mechanisms which may themselves also have consequences for other aspects of behavior (Greenfield and Childs 1991). Although RSCs may not turn out to be unitary, they can still provide a useful starting point for analysis both "upward" by enabling us to understand complex aspects of human behavior and "downward" as starting points for analyzing the neural and endocrine mechanisms underlying behavior.

The Importance of the Sociocultural Structure

It may be useful to consider one example of the importance of the sociocultural in human development within one particular society. In a study of 4-year-olds, no difference was found in average shyness between boys and girls. But there were dramatic differences in the correlates of shyness. Little boys who were shy got on less well with their parents and siblings, and less well with teachers and peers at school, than nonshy boys, but exactly the opposite was true for girls. (It is important to note that the sample did not contain children who were extreme on shyness.) Comparable data have been obtained in Bethesda by Radke-Yarrow, Richters, and Wilson (1988). Interview material suggested that the differences stemmed from the value systems of the parents—they considered shyness to be a virtue in girls but deprecated it in boys. Clearly, this could have considerable implications for later development (Stevenson-Hinde and Hinde 1986).

An Interdisciplinary Issue

We must ask how these differences between boys and girls arise. The considerable controversy to which the nature of gender differences has given arise in recent decades can, I believe, be defused if we distinguish clearly between a number of questions which are logically distinct and require answers from different scientific disciplines:

- How do the differences develop? As we have seen at this meeting, the answer here involves the action of hormones on brain development prenatally, and it also depends on later processes of socialization (differential reinforcement, modeling, etc.). In the latter, the child's caregivers are guided by the gender stereotypes of the culture.
- Why are the differences in a particular direction? The direction of the differences is similar in virtually all cultures, and the answer here is biological. A variety of lines of evidence shows that the direction of the differences between the sexes is such as would have maximized individual reproductive success in the environments in which our early hominid ancestors lived.
- Why are the differences as large as they are and patterned as they are? Here the answer is psychological and concerns a number of mechanisms by which the behavior of children and adolescents tends to accentuate the differences between the sexes. Thus, young children tend to split into single sex groups and to denigrate members of the opposite sex. Later, adolescents strive to accentuate in themselves those characteristics which they believe will be attractive to the opposite sex.
- Why does the extent and patterning of the differences differ between cultures? Here the answer must be anthropological and concerns the tendency toward a degree of congruence between the different aspects of a culture.

Further discussion of these issues can be found in Alexander (1981), Buss (1994), Hinde (1991*a*), and Short (1979).

Analyzing Complex Human Behavior in Terms of RSCs

As a somewhat extreme example of the manner in which complex human activities can be understood in terms of relatively stable characteristics, we may consider the case of international war.

Soldiers fight in wars primarily because it is their duty to do so: aggressiveness plays little part in modern war. Indeed, aggressiveness does not cause war, wars cause aggression. International war is in fact an institution, with numerous constituent roles (soldiers, generals, politicians, munitions workers, medics, and so on), each with its constituent rights and duties.

Since international war is horrendous and against the personal interests of nearly all those taking part, one must ask what maintains this institution as part of our sociocultural structure? The forces that support it can be found at many levels, from matters of everyday life (e.g., novels that sanitize and glorify war), through pervasive cultural issues (e.g., nationalism, some sorts of religious teaching), to the military-industrial-scientific complex. Each of these involves complex dialectics with basic human propensities. For instance, the military-industrial-scientific complex is maintained in part by the career ambitions of those involved, and these stem from basic human needs to cope and control, channeled by relationships with others and by the sociocultural structure.

To take a more specific example, consider the nationalist propaganda used, especially in time of threatened or actual war, to enlist support for the war effort. This often portrays the enemy as evil, dangerous, and even subhuman. It plays on a number of human propensities (RSCs)—for instance, fear of strangers (a basic propensity that first appears in the second half of the first year and may remain in some degree throughout life), fear-induced aggression, and group solidarity (Hinde 1991*b*, 1993; Hinde and Watson 1995).

Conclusion

In conclusion, specialization within the behavioral sciences, though both necessary for progress and inevitable, involves a tendency to investigate particular problems at only one level of complexity, and this can bring only partial understanding. Relatively simple aspects of behavior, such as fear of snakes, may depend in part on, and contribute to, aspects of the sociocultural structure. Enormously complex aspects, such as international war, rest on, and affect, basic human propensities. Full understanding can be achieved only if one is prepared to disregard disciplinary boundaries and come to terms with the dialectical relations between levels of complexity by crossing and recrossing between them. The identification of relatively stable characteristics may provide an heuristically useful starting point for understanding both the bases of complex human activities and the integration of neural and subneural mechanisms.

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Variability in the Effects of Experience on the Development of Cerebral Specializations: Insights From the Study of Deaf Individuals

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ABSTRACT

This chapter reviews our ERP and fMRI results during visual processing and language processing from adults who have had different early sensory and/or language experience. These studies suggest that within vision and within language different neural systems display considerable variability in the degree to which they are modified by early experience. Within vision, early auditory deprivation has most marked effects on the organization of systems important in processing motion information. The results raise the hypothesis that the dorsal visual system displays greater developmental plasticity than does the ventral visual pathway. In addition, different subsystems within language display varying degrees of modifiability by experience. The acquisition of lexical semantics appears relatively robust and invariant even in individuals with markedly different timing and modality of language input. By contrast, systems active during grammatical processing display marked effects of alterations in the timing and nature of early language input. These results converge with other lines of evidence that suggest it is important to distinguish these different language systems. Different accounts for these differential effects of early experience on subsystems within vision and language are discussed.

The issues around which this conference has been organized are central to each of us, not just as scientists but as citizens, parents, and educators. Elucidation of the role of experience in neural and behavioral development is central to a basic understanding of how the nervous system develops for adaptive function. Additionally, research along these lines carries important

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implications for the design and the timing of programs of education, habilitation, and rehabilitation.

Everyone acknowledges that there is an important role for the genotype in human development and that experience is essential in the expression of that genotype. A key issue within neuroscience and psychological science is to specify the degree to which the genetic constraints limit the scaffolding and to know the degree to which these constraints can be modified and the times in human development when they can be modified.

Here we summarize research on the effects of early sensory experience and early language experience on the development of the human brain. The main point we wish to emphasize, based on results from several different studies, is that there appears to be considerable variability in the degree to which different brain and behavioral systems are modified by early experience.

The mechanisms responsible for this variability in experience-dependent changes in the human brain are not yet understood. They may be mediated by differences in maturation rates, in the initial degree of overlap of connectivity between and within different brain regions, in the degree and timing of inhibitory mechanisms in the different brain regions, or perhaps, by differences in the overproduction and pruning of synapses and receptors in different brain regions. The variability in experience-dependent changes in human development can be important in helping to identify different subsystems and in specifying the timing when different kinds of subsystems can be responsive to and modified by input from the environment.

We have compared different and specific aspects of cerebral organization in normal adults and in adults who have had specific alterations in early experience. Normal-hearing adults and congenitally deaf adults were compared to assess the impact of the absence of auditory input on visual development, and also to assess the effects of deaf subjects' altered language experience on the development of the language systems of the brain.

We hypothesized that distinct functional subsystems within vision and language may display different degrees of modifiability following different early experiences that may depend on the rate of maturation of the various neural systems involved. For example, visual processes that arise within the retina mature quickly and display a short period during which they can be modified by experience ("sensitive period"). By contrast, binocular functions that rely on later developing cortical neurons display a considerably longer sensitive period.

In our research, the physiology of sensory and language processing in humans is studied using two brain-imaging techniques: event-related brain potentials (ERPs), and functional magnetic resonance imaging (fMRI). Both of these techniques involve recording indices of brain activity while subjects perform different visual or language tasks. ERPs are voltage fluctuations in the electroencephalogram extracted by signal-averaging techniques. The latency of different positive and negative components in an ERP reveal the time course of the activation (within microseconds) of the underlying neural populations.

The distribution of ERP activity between and within the hemispheres is determined by the anatomical position and geometry of the contributing neurons. The spatial resolution of the ERP can be enhanced by transforming voltage maps of electrical activity to current source density maps that provide a reference-free estimate of the instantaneous electrical currents flowing from the brain perpendicular to the scalp at each location at the specified time point. Thus, ERP recordings can provide exquisite information about the timing of sensory and language processes as well as constraints on their location.

The fMRI technique permits the monitoring of the local increase in oxygen delivery that occurs in neurally active cerebral tissue. As the ratio of oxygenated to deoxygenated hemoglobin increases within the microvasculature of metabolically active areas, an increased MR signal relative to the resting state is observed from these areas. This noninvasive technique has an exquisite spatial resolution (a few mm).

Visual Processing

Although there is considerable anecdotal evidence that deprivation of input in one sensory modality leads to compensatory increases in the functioning of the remaining modalities, the pertinent human behavioral literature presents a confusing array of results. Some studies report increases, some report decreases, and others no differences in the abilities of remaining modalities compared with those abilities in intact control subjects. This variability may be due in large part to differences in the age of onset, completeness, and etiology of the sensory deprivation (deafness or blindness). For these reasons, our studies have focused on deaf individuals who were born profoundly deaf due to a genetic etiology in which the central nervous system is not directly affected. These subjects learned ASL from their deaf parents at the same age that normal-hearing children acquire spoken language.

In an early study, we observed that ERPs to peripheral and foveal (i.e., center of the visual field) visual stimuli differed in morphology and distribution over the scalp in normal adults in a manner consistent with the hypothesis that they were generated by different cortical systems. Results from congenitally deaf adults in the same paradigm showed that, whereas ERPs to foveal stimuli were similar in the two groups, ERPs to peripheral stimuli were two to three times larger in deaf than in hearing subjects over superior temporal cortical areas (Neville et al. 1983). We hypothesized that the "transient" visual system, proposed to mediate the processing of peripheral, spatial, and motion information may, through a process of competitive interactions, take over what would normally be auditory cortical fields in primary or secondary auditory areas or within multimodal temporal areas.

To test the generality of these findings, we extended these studies to conditions under which visual attention was required. We tested the hypothesis that attention to central and peripheral visual space is mediated by different neural systems in normal-hearing adults and, further, that the systems important in processing peripheral motion information are more altered by auditory deprivation than are those important in processing central visual information (Neville and Lawson 1987*a,b,c*). ERPs were monitored while subjects focused their eyes straight ahead and attended to a white square presented either in the periphery or in the center of the visual field. Subjects' task was to detect the direction of motion of the square.

ERPs elicited by peripheral visual stimuli displayed attention-related increases that were several times larger in deaf subjects than in normally hearing subjects. Also, the attention effects were distributed differently in the two groups. In particular, while for the hearing subjects the principal effects of attention occurred over the parietal region contralateral to the attended visual field, in deaf subjects the effect was also observed over the occipital regions of both hemispheres. Moreover, deaf subjects also displayed considerably larger attention-related increases over the left temporal and parietal regions than did the hearing subjects.

This specific pattern of group difference can be considered with respect to anatomical and physiological evidence from the animal literature, which shows two major types of change can occur following unimodal sensory deprivation from birth. First, there is evidence of increased growth and activity of the remaining sensory systems. The bilateral increase of attention-related changes in occipital regions of the deaf may represent this type of change. Second, there is evidence that the brain systems that would normally subserve functions that are lost—audition and auditory language skills in the case of deafness—may become organized to process other information from remaining modalities. The larger activation within the left hemisphere may represent this type of change. In addition, performance results show that these changes carry functional significance. Deaf individuals were faster and more accurate than hearing subjects in detecting the direction of motion of the peripheral stimuli.

Studies of hearing subjects have shown that visual analysis of a scene relies on at least two main processing streams that are anatomically quite separate. The "what" pathway projects from primary visual cortex to the temporal lobe and is specialized for the identification of objects and the processing of color and of fine visual details, while the "where" pathway projects to the parietal cortex and is specialized for the perception of motion and for the localization of objects. In view of our earlier results, we recently investigated the hypothesis that auditory deprivation primarily alters processing along the "where" visual pathway.

In order to investigate this issue, Armstrong, a graduate student in our laboratory, recorded ERPs to stimuli designed to selectively activate either the ventral visual pathway (high spatial frequency, colored gratings that occasionally changed color) or the dorsal pathway (low spatial frequency, grey gratings that occasionally displayed apparent motion). In response to the color changes, ERP responses from hearing and deaf subjects were similar (figure 1*a*). By contrast, motion-elicited ERPs were significantly enhanced in the deaf as compared to hearing subjects (figure 1*b*) (Mitchell et al. 1997). These results suggest that there is considerable specificity in the effects of auditory deprivation on the processing of visual information. Current source density analyses of these data suggested that there were additional visual generators within temporal cortex of the deaf subjects that were not apparent in the normal-hearing subjects.

Recently, we have begun to use fMRI to try to specify precisely where in temporal cortex additional visual processing may be occurring in deaf subjects. This information will be valuable in determining the mechanisms whereby what is normally auditory cortex could come to process visual information. Preliminary results to date suggest that motion (produced by a series of concentric

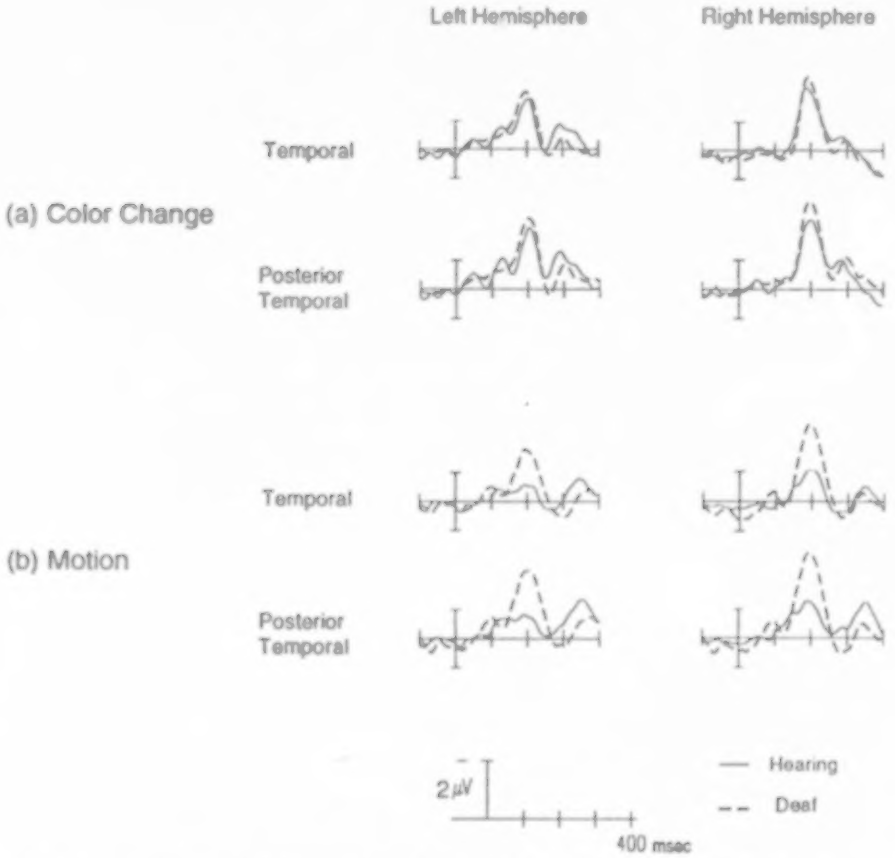

Figure 1 ERPs elicited by *a* color change and *b* motion in normally hearing and congenitally deaf adults. Recordings from temporal and posterior temporal regions of the left and right hemispheres.

rings that expand and contract) activates posterior temporal and occipital brain areas in both normal-hearing and congenitally deaf subjects. However, deaf subjects also display activation within the lateral fissure, that is, where primary and secondary auditory areas are located in normal-hearing subjects. Further studies are needed to establish this pattern and to determine whether the activations are within primary, secondary, or tertiary auditory areas.

One mechanism whereby such changes might have occurred is by the stabilization of what are normally transient connections between early visual and auditory areas. This type of intermodal redundancy has been observed in newborn kittens and hamsters (Innocenti and Clark 1984; Dehay et al. 1984; Frost 1990). It is possible that when auditory input is absent, visual afferents could become stabilized in what would normally be an auditory brain region. Studies of individuals who became deaf after birth suggest the redundancy may exist only during the first 4 years of life (Neville 1990). Ongoing studies of infants and children are investigating this hypothesis (e.g., Neville 1995).

Language Processing

It is reasonable to assume that the rules and principles governing development of the sensory systems also guide the development of cognitive and language-relevant brain systems. As for vision, there may exist biological constraints on the forms of natural language and the organization of the brain systems that mediate natural language. The study of deaf individuals whose first language is American sign language (ASL, i.e., a visual-manual language) and their comparison with native English speakers offers a unique opportunity to assess the idea that there exist biological constraints on the organization of natural languages and that these are independent of the modality through which the language is acquired or the structural characteristics of the language.

Theoretical considerations, formal structures, and behavioral data in adults and children all support the claim that ASL is a fully developed natural language just as are English and French (Klima and Bellugi 1979). ASL exhibits grammatical structure at all linguistic levels including phonology, morphology, and syntax. Grammatical properties that hold for spoken languages are also found in sign languages. Furthermore, children acquiring ASL as a native language from their deaf parents go through the same stages at the same ages as hearing children acquiring a spoken language.

These results suggest constraints on the organization of all natural languages that operate independently of the modality through which language is acquired. At the same time, the modality of transmission clearly affects other aspects of language acquisition and processing. For example, the modality of the language affects the nature of the grammatical devices that the language exploits. In particular, within signed languages, morphological and lexical information is most often conveyed concurrently, reflecting the capacity of the visual system to process information simultaneously. Grammatical distinctions are often conveyed by planes of signing space and spatial loci within these planes (figure 2).

The pattern of similarities and differences implies likely parallels in the identity and operation of the neural systems that mediate spoken and signed languages, but some differences are likely as well. In particular, while aural-oral language processing has been consistently associated with the perisylvian cortex within the left hemisphere, studies of visual-spatial functions in hearing subjects indicate that this type of processing is mediated by parietal cortex primarily within the right hemisphere. The observation that ASL relies on visual-spatial processing at most of the different stages of linguistic analysis raises the hypothesis that the right hemisphere and/or parietal structures participate to a greater extent when subjects process ASL.

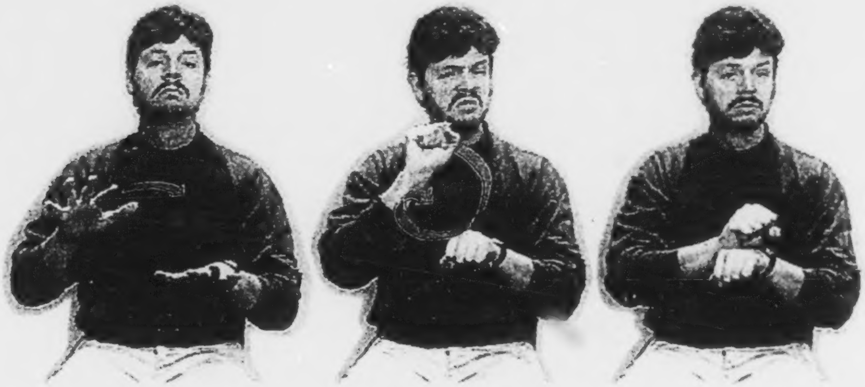

Figure 2. The ASL sign "APPOINTMENT" (arrow indicates motion).

One approach to this issue has been to compare the effects of damage to specific brain regions on particular aspects of oral and sign language production and comprehension. The initial studies along these lines reported similar patterns of hemispheric asymmetries and similar patterns of anterior-posterior dependencies in language breakdown following cortical damage in speaking and signing patients (Poizner et al. 1987). More recently, additional studies have reported discrepancies between the findings of aural-oral and sign language breakdown (Corina in press).

To further assess this issue, we have been comparing cerebral organization for language processing in normal-hearing, monolingual, native English speakers, and congenitally deaf, native ASL signers. ERPs were recorded while subjects either read English sentences or viewed ASL sentences. It is commonly accepted that language processing is decomposable into separate subsystems including semantic (e.g., the meaning of nouns and verbs that refer to specific objects and events) and grammatical processing (structural or relational information) provided in English primarily by words such as articles, conjunctions, and auxiliaries.

In normal-hearing adults processing their native language (English), open-class words (nouns and verbs that refer to specific objects and events) elicited ERPs that were characterized by a negative component that became maximal approximately 350 ms after the word onset (N350) and was largest over the posterior regions of both hemispheres. In contrast, ERPs to closed-class words (articles and prepositions that convey grammatical information) displayed a negative potential largest at 280 ms (N280) that was localized to anterior temporal regions of the left hemisphere (Neville et al. 1992). When deaf individuals processed ASL, they, just like hearing people processing English, displayed more negative ERP responses to open-class signs over posterior regions, while for closed-class signs, the ERP responses were more negative over anterior regions.

This similarity of anterior/posterior ERP pattern as a function of word/sign class membership is consistent with the proposal that significant overlap in the identity and organization of the neural systems within a hemisphere mediates the processing of all formal languages, independently of the modality through which they are acquired. However, while in hearing subjects the ERPs to closed-class words were strongly lateralized to the left hemisphere, in deaf subjects they were symmetrical, suggesting a greater contribution of the right hemisphere during ASL processing (Neville et al. 1997).

In a followup study, we used the fMRI technique to characterize more precisely the brain structures that mediate language processing in these populations. We compared hearing and deaf individuals as they read English sentences and viewed ASL sentences (figure 3, page 18). Consistent with our ERP results, when native signers viewed ASL sentences, robust activation was observed within classical language areas of the left hemisphere, as observed when native speakers of English read English sentences (Neville et al. 1998). These results imply a strong bias for these regions to process language independently of the modality of the language. At the same time, however, native signers, but not native speakers, displayed robust and extensive activation within the right hemisphere. These results are consistent with the idea that the early acquisition of ASL leads to an increased role for right hemisphere structures in language processing. This may occur in response to the important role of visual-spatial information in processing ASL.

The comparison of the processing of written English in deaf and hearing subjects provides an important opportunity to study sensitive periods during development. While hearing subjects learn English from birth, deaf individuals are introduced to English much later (in the group we studied, ASL was the first language; English was the second language, and it was learned late).

ERPs recorded while deaf and hearing subjects read English sentences suggest that semantic and grammatical processing are differentially vulnerable to altered early language experience (Neville et al. 1992). The deaf subjects displayed ERPs to open-class words and other semantic information in English that were similar to those observed when normal-hearing subjects process

English. These results suggest that aspects of semantic processing are robust following deaf subjects' altered early language experience. In contrast, deaf subjects' ERPs to closed-class words that carry grammatical information were markedly different from those of hearing subjects reading the same sentences. The ERPs of deaf subjects lacked the negative (N280) potential over anterior regions of the left hemisphere and did not display any evidence of left hemisphere advantage (figure 4 page 19).

These results are in accord with the idea that language experience has different effects on the development of the several different brain systems that mediate language. Brain systems that mediate grammatical aspects of language processing appear to be more sensitive to altered language experience. This idea is supported by our observation that deaf subjects whose grammar skills were excellent displayed an N280 response that was prominent and asymmetrical just as in normal-hearing subjects (Neville 1991).

Further evidence along these lines comes from the study of bilinguals who acquired English at different ages. Early learners of English showed a large asymmetrical response to grammatical information, while in late learners, this response is altered (Weber-Fox and Neville 1996). Similarly, in an fMRI study comparing hearing and congenitally deaf individuals processing English, we observed marked differences in the brain systems activated in the two groups. Whereas hearing subjects displayed the characteristic left temporal asymmetry, deaf individuals had very little left temporal activity but robust temporo-parietal activation within the right hemisphere (Neville et al. 1998).

The results from the language studies taken as a whole support the hypothesis that there are constraints on the organization of the neural systems that mediate formal language independently of the modality through which language is acquired. The biases include different specializations of anterior and posterior cortical regions in aspects of grammatical and semantic processing. However, it is clear that the nature and timing of sensory and language experience significantly affect the development of the language systems of the brain. Deaf subjects showed an increased role for the right hemisphere when processing ASL and a lack of left hemisphere specialization for grammatical processing of English. These results suggest that functions such as the acquisition of grammar that depend on the perception of rule-based invariances are maturationally constrained and display distinct periods in development when they require specific types of environmental inputs.

Conclusions

The study of cortical organization for visual and language processing in deaf individuals provides support for the concept of different subprocesses with different developmental timecourses and developmental vulnerabilities. The characterization of such maturational constraints on developmental plasticity can contribute to fundamental descriptions of the architecture of different cognitive systems. This information will also contribute to the design of educational and habilitative programs for both normally and abnormally developing children.

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Recovery From Profound Early Social Deprivation

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ABSTRACT

What are long-term consequences of early social deprivation? Are these consequences on a continuum ranging from mild to profound, or is it in the nature of humans to recover from all but the most profound levels of such deprivation? These two questions represent points of departure for a presentation that reviews the findings of several longitudinal studies examining the relationship of early experience to later psychological and social adjustment. When results of this modern research are placed in the historical context of wisdom accumulated over several centuries, a compelling argument can be made that permanent social deficits do indeed result from social deprivation experienced in the first 2 years of life.

We use an ongoing study of extreme social deprivation experienced by institutionalized infants in Romania to explore the second question, which concerns the nature of the behavioral and biological mechanisms that undergird these persisting deficits. The study incorporates both a randomized experiment of early enrichment and a postintervention followup in which institutionalized children are compared with children from the same community who have been raised in families. Measures of physical growth and psychological development are examined in relation to developmental aspects of hypothalamic-pituitary-adrenal regulation.

Implications of this work are formulated in two parts. The first relates to the notion of developmental plasticity. To what extent do the so-called "plastic" elements relate to functions that emerge naturally in the first year of life but require stimulation and support to be sustained, as opposed to functions that are primarily acquired through instruction. The second implication concerns the design of early prevention and health promotional strategies that we view as a foundation for a national program of mental health. Many current intervention strategies appear to exaggerate skill

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development exercises over those that recognize the primacy of emerging social functions in the first 2 years of life. Thus, the potential exists that earnest efforts to provide institutional environments for infants and toddlers may result in various degrees of social deprivation. This makes the need to develop improved systems of monitoring children's early social development highly desirable. We conclude by suggesting ways in which knowledge of developing neural and endocrine systems may enhance current methods of routinely evaluating growth and development in the first 2 years of life.

What are the short- and long-term consequences of early social deprivation? Do these consequences fall on a continuum from mild to profound, or is it in the nature of humans to recover from all but the most severe forms of deprivation? Are some types of cognitive, emotional, or social functions more likely to be permanently affected than others? These questions represent points of departure for a series of investigations, still in an early stage of development, in which we are currently engaged. We are particularly interested in capturing early developmental influences that are the consequence of institutionally based childcare arrangements. These types of experiences have become increasingly normative in the rearing of children. If the family environment constitutes the most efficient system for making human beings human, then the modern world is suddenly challenged with the need to arrange an effective surrogate for the family unit.

Our approach aims to consolidate behavioral, emotional, and physiological evidence in an effort to characterize the impact of these environments on the well-being of infants and young children. To begin this work, we are studying institutionalized infants in state-run orphanages in Romania. We reported on some initial findings from this research in a recent conference on the Neurobiology of Affiliation, sponsored by the National Institute of Mental Health and New York Academy of Sciences (Carlson and Earls 1997). In this chapter, we place this study in the context of this conference on developmental brain plasticity by examining our general knowledge about recovery from early social deprivation based on studies of animals and humans.

History of the Scientific Evidence

One might choose from a number of sources for historical encounters that bear on the question of recovery from early social deprivation. There are many accounts of efforts to socialize children who were reared "in the wild." The most systematic of these was described by Jean Itard 200 years ago (Itard 1802). In an important way, Itard's work contributed to the origins of developmental psychology. His findings, given a painstaking effort to humanize a child captured from the forests near Aveyron, were disappointing and negative. This child learned many social customs and acquired some language, but his capacity to develop an abiding and affectionate relationship with another person had been lost. Eventually, the child escaped the tireless efforts of Itard and lived a marginal existence.

A century and a half later, knowledge about recovery has grown considerably. The impact of social deprivation that results from brief as well as prolonged parental separations is now well understood. Some hallmarks of this scientific odyssey are exemplified in the work of Bowlby and Harlow, though many scientists have contributed to a vast literature. Harlow's experimental manipulations of the social experience of infant rhesus monkeys should be counted among the most significant studies of this century (Harlow et al. 1965). Longlasting and probably permanent deficits in social and reproductive behavior were produced in these experiments by restricting the type of sensory experiences gained during routine maternal care. Tactile, or contact, deprivation was crucial in the production of durable social deficits. Harlow's astute observations led him to appreciate that the "social mind" was more affected than the "intellectual mind" by the experimentally induced severe deprivation. While his work was especially provocative because it adopted a primate species for its demonstration, equally pioneering work by Levine, Denenberg, and others was being conducted using the rat as a model (Meaney et al. 1991).

During the same decades that Harlow and colleagues were unraveling the story of psychological and physiological damage inflicted by social deprivation in animals, Bowlby, Goldfarb, Spitz, and many others were examining the effects of deprivation experienced in institutional settings on human infants (Frank et al. 1996). Much of this knowledge was conveyed by Bowlby (1951) in a report to the World Health Organization. This body of evidence led to improvements in the quality of caretaking practiced in orphanages and intensified efforts to find alternative arrangements, such as foster care, that represented a better approximation to the biological family unit.

More recently, investigators have systematically compared the efficacy of the rearing environments offered through foster care and formal adoption to those provided by biological parents. A cardinal finding of this work is reminiscent of Itard's results. Once infants have lived in institutions for 2 years or more, only partial recovery of normal social behavior takes place (Hodges and Tizard 1989). Adolescents adopted into secure and supportive homes after spending their first 2 years in an institutional setting fail to form close friendships with peers and prefer adults to age mates in routine social encounters.

Ceausescu's National Laboratory

An interesting historical footnote to Harlow's remarkable studies recounts the role of serendipity in scientific discovery. While attempting to increase the breeding of laboratory-reared monkeys using inanimate surrogates, he observed the infants' preference for tactile contact and clinging to a soft surface over other forms of stimulation. The revelation that this "drive" seemed to rival the hunger drive is what set in motion the series of experiments on the psychological consequences of early social deprivation. Along with the accumulated knowledge from human studies compiled by Bowlby, the information conveyed through this research provided a sturdy foundation for a social policy that nearly eliminated residential institutions for infants in much of the world.

But it was not sufficient to prevent an entire nation from succumbing to the perverse policies of the Ceausescu government in Romania. What started as efforts to rapidly industrialize the nation in the 1960s, eventuated in a set of pronatalist policies that legislated multiple births for women and prohibited abortion and contraceptive methods. Along the way, the government banned the teaching of the social sciences and eliminated the practices of psychology and social work. The mission was part of a national experiment to produce a cadre of workers to "lift" the country to a level of industrialization that would reduce its dependence on the Soviet economy. The resulting coercive policies succeeded numerically but failed miserably in producing the desired workforce. Tens of thousands of infants were given over to the state and placed in dismal institutions because their parents did not have the resources to care for them. Many other features of this national effort blundered, which eventually sparked the December revolution of 1989.

At that moment, the outside world discovered the ghastly conditions of these state-run orphanages, the Leagane. Many international organizations attempted to provide some relief and support to these institutions, and many Romanian children being raised in these settings were adopted by families from outside the country. But these efforts still left the majority of the more than 100,000 children residing in profoundly depriving institutional settings.

This was the situation we encountered in 1994 when we began our research on recovery. In our effort to insert science into this destabilized context, our agenda was to combine an ethical response to the blatant violation of the rights of Romanian infants with a research question regarding how best to monitor and evaluate efforts to restore normal development in these deprived children. These aims were complicated by the fact that many of the professionals responsible for management of these institutions had themselves received poor training in psychology and other social sciences. Perhaps it should not be a surprise given these circumstances to find that the medical care and nutritional status of these infants was far better than the psychological care they received and their social-emotional status.

Development of the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Our questions relate specifically to development of the infant's physiological capacities to regulate stress associated with social deprivation. Two complex, multilevel neuroendocrine systems mediate the body's response to acute (and chronic) stress. The locus ceruleus-norepinephrine sympathetic nervous system (LC-NE-SNS) axis is the rapidly responding component, which also stimulates release of epinephrine (EP) from the adrenal medulla. The HPA axis is the more slowly responding component of the generalized stress response, comprising the hypothalamus (and corticotropin-releasing hormone and arginine vasopressin, CRH and AVP) and the pituitary gland (and the adrenocorticotropic hormone, ACTH), which stimulates the release of the glucocorticoids, GC (cortisol in humans and other primates and corticosterone in rodents), from the cortex of the adrenal gland. These neural and hormonal factors exert powerful influences on many organ systems, including the brain, which are adaptive under conditions of acute stress but become pathological under conditions of chronic distress (Chrousos and Gold 1992).

The relationship between the neural and behavioral effects and chronic stress is best understood for the HPA component of the generalized stress response. Regulation of the basal levels of circulating GC has been examined in relation to the diurnal sleep/wake cycle as well as in response to physiological or psychological sources of stress. Characteristically, the basal level of cortisol secretion in humans rises prior to waking and declines throughout the waking hours. This diurnal regulation of basal cortisol levels is believed to depend upon one class of cytosolic GC receptors (Type I), found in the hippocampus, and the HPA axis, which controls the level of synthesis and secretion release of CRH, AVP, and ACTH (Dallman et al. 1987, 1989). Developmental studies of the regulation in human infants show a two-phase pattern in adrenocortical activity in the first 2 months of age, with an adult-like circadian pattern emerging at around 3 months, coincidental with daily patterns of sleep and feeding (Spangler 1991; Price et al. 1983)

HPA reactivity to en acute stressor is characterized by a CRH/AVP-ACTH-GC cascade occurring within minutes of an acute stress event, showing a combined fast- and slow-phase of recovery over the next 15-45 minutes. The regulation of cortisol secretion in response to stress appears to depend on both classes of GC receptors (Type I and II) found in the hypothalamus, pituitary, and hippocampal formation. In response to high circulating levels of GC, these receptors appear to play a key role inhibiting or constraining activity at all levels of the HPA axis (Suchecki et al. 1993; Bradbury et al. 1994). During the first months of life, the HPA axis of human infants is particularly labile. But the amplitude and duration of the HPA response begins to come under better regulatory control between ages 6 and 12 months (Gunnar et al. 1991).

In addition to the expected developmental changes occurring in endogenous GC receptors in hippocampus and HPA axis (based on research in rodents), it is important to consider the role of exogenous "social buffers" and the powerful effects that caretakers and companions exert on the stress response of humans and other primates when faced with novel and threatening situations (Hertsgaard et al. 1995; Gunnar et al. 1992). In fact, the removal of a young organism from its social "buffer" constitutes one of the most powerful elicitors of activity in the HPA axis. If a rat pup is removed from its mother and home cage for even a brief period (e.g., 15 min), the mother will increase tactile stimulation of pups upon reunion. This manipulation, known as handling, has been repeatedly shown to result in handled pups showing less fearfulness and better regulation of the HPA responsivity, along with an increase in GC receptor binding sites in hippocampus and frontal cortex, but not in the pituitary, hypothalamus, septum, or amygdala (Meaney et al. 1985; Rosenfeld et al. 1991).

By comparison, nonhandled or chronically stressed rodent pups show downregulation of GC receptors, resulting in less effective containment of HPA responsivity and chronically elevated levels of circulating GC in response to environmental events (Bodnoff et al. 1995; Meaney et al. 1989). Vigorous tactile stimulation beyond the preweaning period in nonhandled rodents does not compensate for early deprivation, indicating a "critical period" for this effect (Meaney and Aitken 1985). Other forms of prolonged and intense stress as well as endotoxin exposure have been shown to yield decreased GC receptor number in hippocampus and frontal cortex of rodent pups during the preweaning critical period (Shanks et al. 1995).

These nonhandled rats also show accelerated loss of hippocampal neurons at maturity, which, in turn, is accompanied by varieties of memory impairment that are contingent on hippocampal functions. Recent studies of elderly adults show a similar relationship between increased HPA reactivity and premature memory loss (McEwen and Sapolsky 1995).

An Experiment to Promote Recovery

Research in socially deprived infants living in institutions in Romania was prompted by the consistency of clinical and laboratory findings on the dire consequences of social deprivation. A wave of international disbelief and condemnation was evoked by the revelation, in early 1990, of the conditions of hundreds of thousands of infants and children living in residential institutions in Romania. Although UNICEF and other international agencies and organizations have provided financial and technical support for these children, the rate of admission remains as high today as under Ceausescu's severe pronatalist policies.

As in many Eastern European and former Soviet countries, the transition to democratic rule and a market economy threatens the stability and integrity of communities and families that traditionally care for infants and children. Today, between 2 and 3 percent of Romanian children are spending their lives in a series of institutions—moving from the maternity home to the "cradles" (or Leagane for ages 0-3) and next to the preschool institutions and children's homes (for children and youth aged 3-18). By age 2 to 3 years, most of these children show mild to severe developmental delay due to the early social deprivation experienced in these vastly understaffed institutions which place exclusive emphasis on custodial and medical care.

Our research involves measuring physical growth, psychological development, and patterns of cortisol secretion as part of a randomized intervention study designed to restore normal development in infants residing in Leagane in Iasi, Romania. The interest in cortisol secretion as an indicator of HPA functioning was to see if the demonstration in rodents that HPA regulation could be irreversibly disrupted by social deprivation in the first weeks of life applied to human infants experiencing early social/tactile deprivation in the first months of life. At birth, the majority of children in these institutions are of normal weight and gestational age but are "placed" because of the poor social and economic circumstances within their families.

The small population with which we worked had been part of a 13-month social and educational enrichment program developed by Sparling and associates (1992), which unexpectedly ended 6 months before our study began. Infants between 2 and 9 months were randomly assigned either to the standard Leagane condition (n=28) or to a social/educational enrichment program (n=30). Although the intervention group had shown significantly greater psychological and physical growth during the enrichment period, 6 months after that period, the mental/motor scores (on Bayley Scales of Infant Development) for the intervention group were not significantly different from those of the controls, and both groups were performing at 50–70 percent of age expectation. Also, indices of physical growth were no longer different for the groups, averaging in the 3–10 percentile by North American and Western European norms. Similarly, the two Leagane groups did not differ in average basal salivary cortisol levels (measured in saliva samples for 2 days in the morning, noon, and evening). The standard groups did show significantly elevated basal cortisol levels compared to the enriched group in the noon period when cortisol levels are usually low (Carlson et al. 1995).

Another indication of HPA dysfunction was the lack of diurnal variation in basal level cycles typical of children at this age. Given the dysregulated daily cortisol levels, it was surprising to find that the elevated average basal levels (as well as most levels at specific times of the day) were significantly correlated with low mental/motor scores and low physical growth measures for the individual groups and for the two groups combined.

Given the differences between the two Leagane groups, we returned in the following year to sample cortisol and obtain psychological measures from preschool children from state-run daycare units (the Crese) to compare with the findings on institutionally reared children. Salivary cortisol samples were obtained from one group at age 2 (n=20) and another group at age 3 (n=21) over a 2-day period (morning, noon, and late afternoon), along with growth indices and performance levels on Bayley Scales. We were astonished to find elevated levels of cortisol in these children (comparable to Leagane-reared children), despite the fact that prior to their enrollment in the daycare programs at age 2, they had being raised exclusively by their parents. Again, those children with the highest average, morning, noon, or afternoon cortisol levels had the lowest mental/motor ranking on the Bayley Scales. In contrast to Leagane children, physical growth indices were not correlated with cortisol values. In contrast to the Leagane-reared children, average Bayley scores and weight indices were not different from US norms.

To gain a longitudinal perspective on the elevated and dysregulated HPA activity in the Leagane children, we did a 13-month followup to determine if HPA profiles had changed in the intervention group after acclimating to the standard Leagane conditions or as the 3-year-old children in both groups moved to preschool institutions. Results from the followup did reveal improved HPA regulation in both the standard and enriched groups in the morning period, but the noon and evening levels were more elevated than in the previous year (Carlson et al. 1997).

These longitudinal studies of the Leagane-reared children have not revealed the benefits of early enrichment on the development of HPA regulation for the intervention group (compared to the standard group) expected from the laboratory findings. However, the benefits of the enrichment program upon psychological and physical development were also lost by 6 months after the program ended. Furthermore, the average age of assignment into the intervention group was 6 months and may have been beyond any critical or sensitive period for the regulation of HPA reactivity. Given that the Leagane children are currently living in depriving conditions, not unlike the conditions in which they spent most of their early years, it is difficult to differentiate how HPA profiles reflect past or present conditions.

The results of a 6-month followup of the Crese children are equally disturbing in that they indicate that basal levels are high during this extended exposure to the custodial daycare settings in which they spend about 40 hours a week. For these Crese children, these levels assume a normal pattern, but remain elevated over the weekend, when cortisol is sampled by the parents morning, noon, and evenings. Again, it is difficult to separate the adverse effect of their exposure to low-quality daycare from the chronic stress they may experience directly or indirectly due to the unstable conditions of that society affecting parents and caretakers.

The correlation between mental and motor performance and abnormal cortisol levels cause us to be concerned that these levels may be having a possible long-term effect on neural and psychological function (McEwen and Sapolsky 1995). These general mental/motor scales may be reflecting a more specific deficit in memory function predicted by laboratory studies (Bodnoff et al. 1995) and seen in adults with elevated cortisol levels associated with depression or Cushing's disorder (Starkman et al. 1992) or with subnormal cortisol levels seen in posttraumatic stress disorder (Suchecki et al. 1993).

Future Directions

The finding of persistently abnormal levels of cortisol in Crese children has motivated us to extend this study in two ways. First, we are interested in studying the transition of formerly institutionalized children to families, either through formal adoption or the creation of alternative strategies adopted through governmental or nongovernmental organizations in Romania. This type of study should improve our understanding of the degree to which recovery in social and cognitive functions does take place and the mechanisms that underlie this plasticity. Evidence already exists that Romanian infants adopted after age 9 months, but not before 4 months, have persisting deficits in later childhood that reflect both poor regulation of social behavior and poor judgment in social encounters with peers and adults (Chisholm et al. 1995). These results echo those of Hodges and Tizard (1989).

The second type of study employs knowledge of HPA physiology to study the quality of daycare environments in the United States. In this country, evidence is mounting that increasing numbers of children are growing up poor (Duncan 1994), and that even middle-class children are being exposed to childcare facilities of poor quality (National Association for the Education of Young Children 1995). It is conceivable that without convincing evidence about the seriousness of these less than profound levels of deprivation, institutional care will deteriorate further, while the proportions of young children encountering these environments continue to increase.

But what constitutes convincing evidence? If not the detailed and passionate description of one child by Itard, the imaginative experiments of Harlow, or the masterful synthesis of knowledge by Bowlby, then what? A fundamental reason for conducting this research is to establish the degree to which information derived from a physiological system such as the HPA enhances our capacity to determine the level of deprivation experienced by the child and sensitive periods in development when deprivation exerts particularly harmful effects.

Based on this work so far, the findings appear promising. We recommend that this general line of inquiry be pursued toward the ultimate goal of promoting the mental health of children (Earls and Carlson 1995; Earls and Carlson 1993). Making the claim that every child has a right to a family and to be protected from socially depriving circumstances raises the prospects for health promotion, but it does not create such a reality. Scientific approaches that directly and sensitively reflect the child's point of view are required to substantiate these claims. Along with data from behavioral observations and cognitive tests, information about the child's physiological adaptation to compromising environmental conditions should constitute a powerful addition to the base of knowledge about early social deprivation. Perhaps then the claims pronounced in documents such as the UN Convention on the Rights of the Child (Limber and Flekkoy 1995) will be sufficiently compelling to change the insecure and unstable environments affecting huge proportions of the world's children.

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The Plasticity-Specificity Conundrum

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ABSTRACT

The prefrontal cortex in adult humans represents one of the most functionally advanced areas of association cortex and the area most often associated with the complex human executive functions of comprehension, thinking, and planning. As the structural and functional organization of this system has long been assumed to be particularly plastic (e.g., in comparison to primary visual cortex), study of its organization, development, and plasticity should provide insight into the cellular mechanisms available to the mammalian cortex in the domain of neural plasticity.

Analysis of the circuitry of prefrontal cortex has revealed that its major cytoarchitectonic subdivisions are defined by connections with sensory, motor, and limbic areas of the brain. These anatomical studies have affirmed that the prefrontal cortex is organized not as a central processor into which all sensory streams converge, but rather as an assembly of special-purpose areas, each dedicated to a different informational domain. A single domain is defined by its distributed multiple interconnections with other cortical areas as well as thalamus and basal ganglia—all components of a network or system integrated by a shared informational domain, for example, spatial, object, or affective cognition. Single-cell physiology in behaving animals has supported the domain specificity of prefrontal function at the cellular level.

How do dedicated networks with specialized functions develop? While a large body of information is available on the behavioral and electroencephalogram development of children, less attention has been paid in the neurosciences to the development of neural systems related to higher cognitive functions. We have conducted studies on the development of specific circuits as well as a detailed quantitative electron microscopic examination of synaptogenesis in the prefrontal cortex of nonhuman primates. Our goal is to understand the timing and kinetics of cortical

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maturation and the relation of synaptic circuitry to the emergence of behavioral capacity.

We examined synapse formation in five distinct cortical areas in more than 25 fetal, neonatal, adolescent, adult, and old-aged rhesus monkeys. Results affirm that (1) synapses are overproduced in all layers of prefrontal cortex; (2) highest density of synapses is reached in all layers between 2 and 4 months of age; (3) rate and tempo of synaptogenesis in prefrontal cortex proceeds concurrently with that in other areas; and (4) adult levels of synapse concentration are stabilized in prefrontal cortex at or around puberty. In all five areas examined, including the prefrontal association cortex, the accretion of D1 and D2 dopamine receptors, as well as SHT1 and SHT2 receptors, parallels synaptogenesis. The Bmax values (density) of neurotransmitter receptors increase exponentially through birth, reach a peak between 2 and 4 months of age, and then gradually fall to adult levels by puberty.

The achievement of peak synaptic and receptor density at 2-4 months of age in rhesus monkeys reflects a hallmark in brain development that has a parallel in the human brain when children are 7½–12 months of age. This is the age in both species when intentionality begins to be expressed and to mature. At the same time, perceptual discrimination, visual acuity, motor control, and delayed responding evolve concurrently in the behavioral repertoire. There is reason to believe that these multiple functions appear in parallel with the concurrent maturation of cortical areas. Plasticity in behavioral repertoires emerges from dedicated circuitry; distributed parts of a given neural system appear to emerge concurrently during development.

Roundtable Seminars

Biobehavioral Profiles Related to Stress Reactivity and Ethnic Variation

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Emory University

ABSTRACT

An important factor in human neurobehavior is the role of mediator and moderator variables in the expression of biobehavioral profiles during early life. To address this issue, preliminary cross-cultural (i.e., Hong Kong Chinese, African American) findings are presented that depict differing patterns of physiologic (hormonal) and behavioral responding. These perinatal profiles generally support earlier research suggestive of early temperament/behavioral style differences between newborns from ethnically diverse populations. It is hoped that these findings will stimulate further discussion pertaining to issues of causation, levels of analysis, and alternative pathways of phenotypic expression of biobehavioral traits.

Individual Differences

Biobehavioral profiles are a salient characteristic of very young organisms due to the prominent role regulatory functions play in early homeostasis. These profiles reflect general and unique characteristics of the individual. Responsiveness to external stressful experiences, along with variations in phenotypic expression, parallels genetic influences that contribute to biobehavioral integrity as indexed by reactivity to environmental challenge. Some of the variation in biobehavioral profiles have been referred to as differences in temperamental style and have traditionally been attributed to selective cultural factors or rearing practices. Empirical studies are beginning to reveal that biological

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factors are important mediators of observed temperamental patterns in infants and children (Goldsmith et al. 1987; Rothbart and Derryberry 1982) and that these biological factors which underlie temperamental predispositions are present at birth (Buss and Plomin 1984; Carey and McDevitt 1978; Thomas and Chess 1977; Emory and Toomey 1991).

One of the prominent features of behavioral style is inhibition, and inhibition of behavior has been related to frontal lobe development, cognition, and affective behavior in human infants during the first year of life (Fox and Bell 1990). The degree of behavioral inhibition seems to be a reliable inter-ethnic difference in temperamental style. Behavioral inhibition is defined as a temperamental construct that reflects a tendency to be shy, timid, and behaviorally constrained in novel or stressful situations (Hirshfeld et al. 1992). Animal studies have shown that behavioral inhibition is a corticosterone-dependent developmental response (Takahashi 1994, 1995). Corticosterone is a hormone activated and released by the hypothalamic-pituitary-adrenocorticoid (HPA) axis is response to stress.

In studies with rat pups, behavioral inhibition has been defined as freezing and a reduction in ongoing locomotor behavior and vocalization in response to stress. The majority of animal studies have placed rat pups in stressful environments and observed changes in their behavior and how these changes relate to HPA activity. Disrupting the developmental action of endogenous corticosterone appears to impair the ontogenic expression of behavioral inhibition such that adrenalectomized rat pups exhibit deficits in this response (Takahashi and Rubin 1993). They show a greater degree of locomotor activity and vocalization when placed in close proximity to an unfamiliar adult male. Moreover, administration of exogenous corticosterone restores behavioral inhibition in adrenalectomized pups (Takahashi and Kim 1995). Further support for the mediating action of corticosterone on behavioral inhibition was evidenced in a study of centrally administered alpha-helical corticotropin-releasing factor, ahCRF, a corticotropin-releasing factor antagonist, on the locomotor activity and vocalizations of isolated guinea pig pups. Hennessey et al. (1992) found that ahCRF enhanced vocalizing and locomotor activity. Thus, several studies support the notion of behavioral inhibition and link it directly to biochemical activity.

With respect to humans, several studies have reported ethnic differences in infants' cortisol response to stressful stimulation which appear to similarly mediate observed differences in behavioral inhibition. Lewis and colleagues (1993) found that Japanese infants had significantly higher salivary cortisol values in response to inoculation than a matched sample of Caucasian-American babies at 4 months of age. Although the Japanese infants had higher levels of cortisol than their Caucasian counterparts, they exhibited inhibited behavioral responses to the inoculation. Japanese infants cried significantly less and showed a shorter latency to quiet after inoculation than Caucasian infants. A similar pattern of responding was observed in response to a pediatric examination (Lewis 1989). Japanese infants, 3 to 5 months old, exhibited higher cortisol values and less distress than American infants in response to this relatively stressful handling manipulation. This pattern of responding has been interpreted as support for Levine and Wiener's (1989) and Suomi's (1991) hypothesis that inhibition of the behavioral response is associated with a greater cortisol response. We suggest that these variations in responsivity to stress among human infants index important components of temperamental style and serve as a phenotypic feature in the constellation of responses that make up the biobehavioral profile.

These results are consistent with the findings of others who have reported differences between Caucasian-American and Asian newborns in various aspects of temperament in the early months of life. Relative to Caucasian-American infants, Asian infants appear to be less labile in state, less excitable, and more readily calmed when upset (Freedman 1974, 1976; Freedman and Freedman 1969; Kuchner 1979; Caudill and Frost 1975; Caudill and Weinstein 1969). For various reasons, much of this research has been dormant over the past 25 years with the notable exception of studies by Lewis and his colleagues.

Working in collaboration with the Chinese University of Hong Kong in a study of infant cortisol responding and postnatal behavior, we have found additional support for the notion of biobehavioral profiles that vary along ethnic lines during the neonatal period.

Method and Procedures

Subjects

A matched sample of 20 healthy African-American newborns (10 male, 10 female), and 20 healthy Chinese newborns were recruited from Crawford Long Hospital of Emory University and the Prince of Wales Hospital of the Chinese University of Hong Kong, respectively. There were no significant differences between the samples with respect to parity, length of labor, gestational age, or birthweight.

Admission Heelstick

The admission heelstick consisted of pricking the infant's heel for the purposes of blood collection. This procedure is routinely performed upon admission to the neonatal nursery. A saliva sample was collected from the infants immediate preceding and 25 minutes following the heelstick. All 40 subjects underwent the heelstick manipulation at approximately 2 hours of age.

Neurobehavioral Examination

A subset of 20 subjects, 10 from the Chinese sample and 10 from the African-American sample, were administered the Neurological and Adaptive Capacity Scale (NACS) for full-term neonates (Amiel-Tison et al. 1982) at approximately 24 hours of age. The NACS consists of 15 items which examine a newborn's adaptive capacity, passive tone, active tone, primary reflexes, alertness, crying, and motor activity. Each item is scored 0, 1, or 2, with higher scores indicative of more highly developed responses.

Cortisol Assay

Cortisol was assayed using the Incstar Inc. Kit for free cortisol in saliva. Assays were performed in batches, with all samples from the same baby in the same assay batch to control for interassay variation. With respect to specificity, cross-reactivates in the salivary assay were prednisolone 83 percent, 11-deoxycortisol 6.4 percent, cortisone 3.6 percent, and corticosterone, 2.3 percent. The intra-assay coefficient was 6.2.

Results

An independent groups t-test revealed that the Chinese infants had significantly higher mean salivary cortisol values than their African-American counterparts both before and after the heelstick manipulation. The mean cortisol values for the two saliva samples are presented in figures 1 and 2.

The significant differences in salivary cortisol were not due to the effects of anesthesia administered to the mothers during labor and delivery. An independent groups t-test revealed no significant differences in the mean salivary

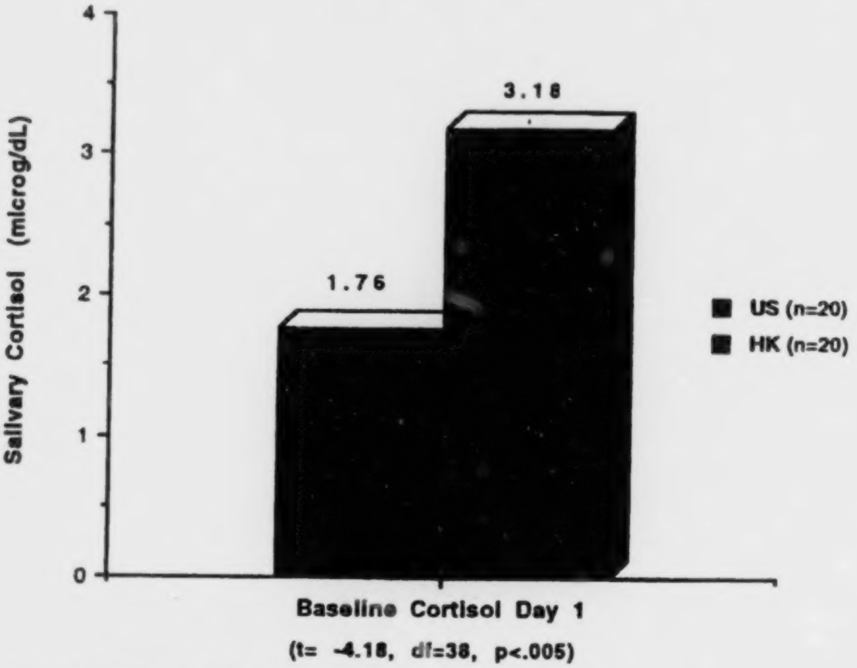

Figure 1 Prestimulation cortisol for heel-stick manipulation

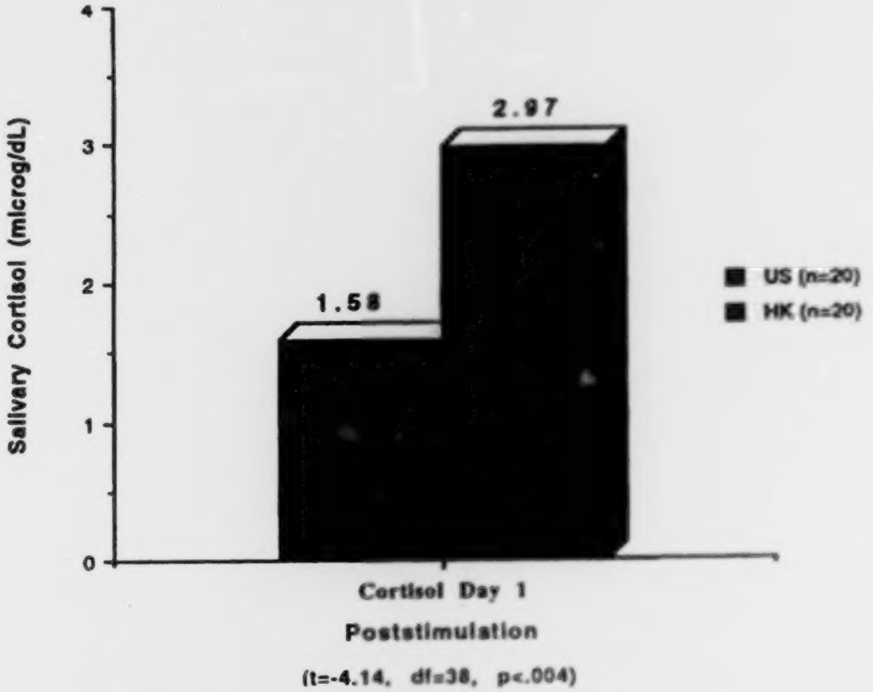

Figure 2 Poststimulation cortisol for heelstick manipulation

cortisol values both before (t (df=38)=-25, p>0.801) and after (t(df=38)=0.67, p>0.508) the heelstick manipulation between infants whose mothers were administered anesthesia verses those who were not administered anesthesia in the delivery room.

With respect to the 20 subjects (10 Chinese and 10 African American) who underwent the discharge-day NACS, the Chinese sample once again had significantly higher mean salivary cortisol values than their African-American counterparts both before and after the heelstick. The mean cortisol values for these two samples are presented in figure 3.

With respect to the neurobehavioral data, although there were no significant differences between the two groups on their total score on the NACS (Chinese=33.4, African American=32.9), there were significant differences on the active tone, adaptive capacity, and primary reflex subscales. A series of independent groups t-tests showed that mean scores on the active tone subscale were significantly higher for the African-American sample than for the Chinese sample. However, the mean scores on the adaptive capacity and primary reflex subscales were significantly higher for the Chinese infants than for the African-American infants (figure 4).

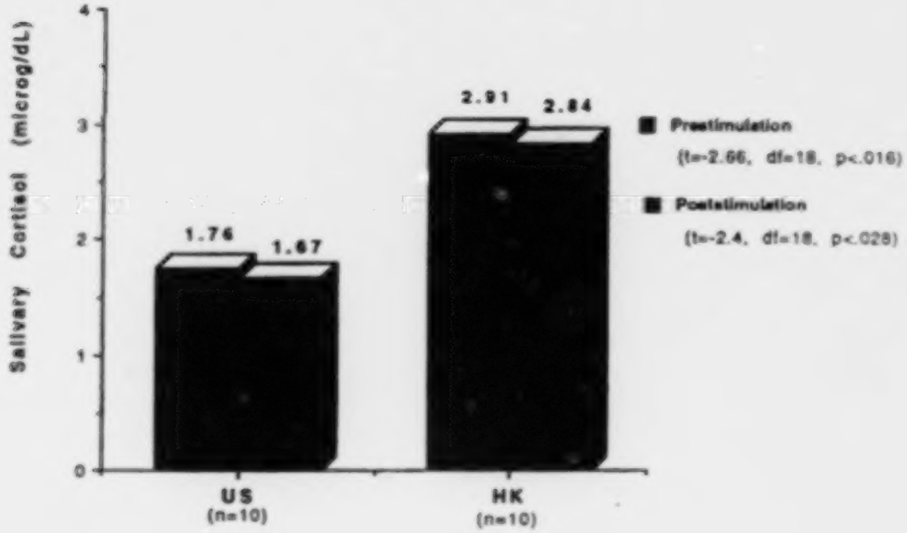

Figure 3 Prestimulation and poststimulation salivary cortisol values for heelstick manipulation

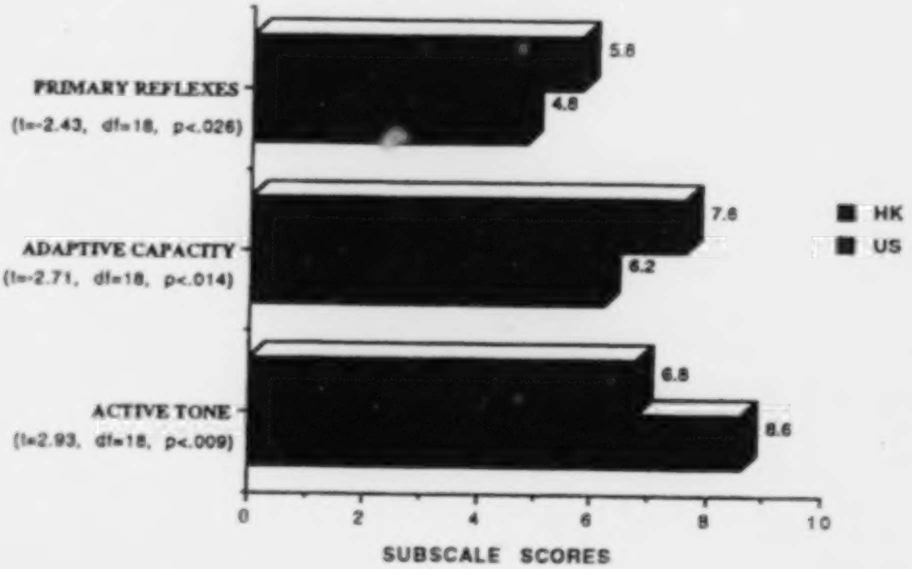

Figure 4 NACS subscale scores

Discussion

Taken together, the cortisol and postnatal neurobehavioral data are highly consistent with the literature and provide additional support for the cortisol-mediated behavioral inhibition hypothesis and the previously reported ethnic differences in temperament.

The heelstick data showed that Chinese infants displayed a greater cortisol response to stress than did the African-American infants. Not only was this difference highly significant, it could not be accounted for by differences in delivery room anesthesia, parity, length of labor, gestational age, or birthweight. Furthermore, the neurobehavioral data are consistent with the literature on ethnic differences in behavioral inhibition in the face of physiological arousal.

The adaptive capacity subscale of the NACS examines the strength of an infant's response to a light and a bell and the subsequent ability to habituate to repeated presentations of these stimuli. The primary reflex subscale examines the presence and the strength of the following reflexes: automatic walking, Moro reflex, and palmar grasp. Our Chinese sample scored higher on these subscales than did their African-American counterparts. As such, the Chinese sample is displaying behavior that is more organized, stable, and adaptive. This pattern of responding is congruent with the behavioral inhibition hypothesis in the sense that lower levels of motor activity might contribute to a more quiescent state which would in turn allow the infant to better attend to its environment and organize its behavioral responses in a more efficient and coordinated manner. Note that the faster rates of habituation in the Chinese sample are congruent with previous evidence which suggested that Chinese babies habituate faster than Caucasian babies. Freedman and Freedman (1969), in a study of Chinese-Caucasian differences at birth, found that when a pen light was repeatedly shone on an infant's eyes and the number of blinks counted until the infant no longer reacted, the Chinese infants tended to habituate more quickly than Caucasian infants.

With respect to the active tone subscale which examined infants' spontaneous activity; active motor control of the muscles in the head, neck, and arms; and response to traction and being pulled to sit, the African-American sample had higher scores than their Asian counterpart. Although these results can be interpreted in light of lower levels of behavioral inhibition in the African-American sample, which would consequently render them more motorically active, they can likewise indicate that the African-American infants are more motorically precocious. The literature suggests that African babies tend to be motorically precocious when compared to Caucasian babies (Gerber 1958; Gerber and Dean 1957). Brazelton and colleagues (1976) have similarly found that African babies are motorically precocious and also reported anecdotal evidence from African-American mothers regarding their motorically precocious youngsters.

What is important about the significant differences among the African-American sample and the Hong Kong Chinese sample is the double dissociation between the two groups in hormonal and behavioral responding. Whereas African-American neonates exhibit much lower cortisol values across conditions (prestimulation versus poststimulation), they have significantly higher behavioral scores on the major item of the NACS that reflects spontaneous motor activity. They also exhibit lower scores on items related to alertness and reflex integrity. In contrast, the Chinese neonates have significantly higher cortisol levels before and after heelstick stimulation but remain behaviorally quiescent while alert and reflexively reactive. Stated more simply, the Chinese neonates' HPA system appears to be in overdrive, while the African-American neonates appear to be in overdrive motorically. The Chinese newborns remain behaviorally inhibited unless externally stimulated, whereas the African-American neonates' HPA system appears to be inhibited.

In conclusion, our data seem to extend the observed ethnic differences in temperament between Asians and Caucasians to include African Americans. They further date the presence of these differences in temperamental style, as evidenced by behavioral inhibition and cortisol responses to stress, at an even earlier age. Whereas previous studies examined the behavior of 3- to 5-month-olds, our study indicated that ethnic differences in aspects of the biobehavioral profile can be detected as early as 2 hours of age. Finally, not only does our data support the claim that biological factors are important mediators of observed temperamental patterns in infants, it also runs counter to the more accepted notion that ethnic differences in temperament are a result of different rearing practices. The early postnatal cortisol and behavioral data provide preliminary evidence of inborn biobehavioral differences along ethnic lines in the newborn period.

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Stress and Early Development: Reflections and Future Directions

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The rich collection of articles drawn from the Stress and Early Development Panel presentations readily illustrates that research programs on stress in the perinatal and early childhood periods are many and varied. Yet alongside this breadth in the arenas of neuroscience, behavioral science, and psychopathological research, gaps also exist in the connections among disciplinary-specific theories and research methods. These integrative connections and dividing gaps were the focus of discussion by the participants in the Stress and Early Development Roundtable.

An example of disciplinary disconnect is found in the variety of ways in which developmental plasticity is defined. For example, Ciaranello and colleagues (1995) suggested that developmental plasticity represents brain function and its subsequent influence on behavior. In this definition, it is noted that the brain possesses self-organizing functions that can, in fact, be altered by experiences at certain sensitive periods of development that occur across the life course. Cicchetti (1993) proposed that developmental plasticity is the shaping of individuals by both genetic and environmental factors, whereby stage-salient unfolding of biological capacities dynamically interact with environmental factors to direct and redirect the course of development. In Thatcher's (1994) description of the theory of cyclic cortical development, he stated that

> iterative growth spurts and patterns of development during the postnatal period reflect a convergence process that narrows the disparity between structure and function by slowly sculpting and reshaping the brain's microanatomy to meet the demands of an adult world. (p. 565)

Each of these definitions mirrors the scientific discipline of its author. However, there are elements common to all. The participants in the roundtable

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presented their own definitions. One stated that developmental plasticity represents the retention or variation of internal events within structures of the developing brain. A second definition focused on taking form during development and maintaining structure at maturity. This acknowledges that the mature organism can still show signs of plasticity, but the challenges are different at different stages of development.

Stress has been characterized by four features: (1) stress is a stimulus event; (2) this event can modify physiological or psychological equilibrium; (3) this disequilibrium is reflected in arousal with neurophysiological, cognitive, and emotional consequences; and (4) these consequences can disrupt or promote individual adaptation (Garmezy and Masten 1990). These features typify stress at any point in the lifespan. However, the consequences of stress may be most salient in the prenatal and perinatal periods. In the exploration of stress during early life, it has been noted that many stressors act synergistically, modifying both physiological and psychological states. For example, maternal alcoholism can stress the fetal brain chemically and then psychologically stress the newborn infant through maternal unavailability, neglect, or abuse. Thus, there are neurobiological and psychological aspects of both the stressor and the response of the fetus or infant. While this example highlights the psychological and physiological aspects of pathological consequences, adaptive coping also may be seen.

In this vein, an overarching challenge was made to the participants in the roundtable to discuss and highlight those ways in which multilevel research in the area of stress and early development might be advanced. In presenting those areas of needed research, the roundtable participants found it useful to consider the common theme of developmental plasticity and the cross-disciplinary subthemes of causation, rates of development, levels of analysis, and alternative pathways.

Causation

The participants noted that future research on stress and early development should include an expanded focus on causation. Two major models of causation—upward and downward—were reflected in discussion, as well as a third, if their interaction is seen as a model system in itself. Most of the extant developmental neurobiological research uses upward causation as its theoretical frame. For example, how do genes and intrinsic physiology direct behavioral development? Similarly, how do genetic, cellular, and physiological interactions cause the development of anatomical structure?

However, much of the work presented at this conference suggests that this approach to understanding development is not sufficient and that future research must consider downward causation as an additional theoretical model. This model presumes that events at higher levels of organization regulate events at lower levels of organization. The incorporation of both models suggests that development of the organism depends upon reciprocally interacting systems. Simple reductions to genetic and cellular levels omit the overall framework within which the organism successfully grows, develops, and adjusts to the constantly changing environment.

There are some examples of the usefulness of the model of downward causation. Emory pointed out that the stressors inherent in the human birth process, from the uterine contractions of labor, through the birth process itself, to the sudden onset of external visual, aural, olfactory, and tactile stimulation, appear to be critical components necessary for lung maturation and even the emergence of such psychologically complex qualities as temperament and personality. Recent work has established that early visual stimulation appears to turn off synapse formation in the superior colliculus. Experiential input drives the development of inhibitory systems, marking the end of a critical period for structure and the beginning of the formation of specific visual sensory processing capabilities (Constantine-Paton, this volume).

Later in life, maternal care and experience can affect the infant's responses to stress, such as adrenal corticoid secretion (which has direct effect on hippocampal neuronal number) and behavioral fear responses that are mediated by other limbic brain regions (McEwen, this volume). Finally, current research suggests that chronic, unresolved anger in the home can have a variety of effects, changing cognitive mechanisms of hypervigilance, which may well be mediated through the amygdala (Cicchetti, this volume).

Thus, genes and intrinsic physiology help direct the behavioral development of an organism, while environmental input (including properties of the individual and of surrounding social structure) is a causal agent in influencing biological mechanisms of development. New work should explore these issues of bidirectional causation in the connections between mother and fetus. Primary considerations include maternal perception and experience of real events and their direction of fetal hypothalamic-pituitary-adrenal (HPA) activity and stress responses. Secondarily, how might the fetus elicit responses in the mother? Researchers also need to further explore parenting as a powerful developmental regulator of social adaptation, biological substrate, and, ultimately, the stress response—following a model where cultural and social factors influence biological state.

There is some evidence for certain cellular mechanisms that mediate the different types of downward causation. For example, the NMDA receptor is an important part of synaptogenesis in the refinement of specific retinal connections (Constantine-Paton, this volume), estradiol effects on sexual differentiation (Woolley and McEwen 1994), and the long-term potentiation effects of learning seen in layers 2 and 3 of the neocortex (Greenough, this volume). However, it is more likely that there is no single mechanism. Future research will determine how large this set of regulatory mechanisms might be.

Rates of Development

Questions surrounding the shifting rates of normal perinatal development deserve more intensive and broad exploration. It has been suggested that fluctuations in the rates of the development of certain subsystems may influence fetus' and infants' susceptibility or resistance to stressors during this period. For example, researchers have postulated that the frontal lobe has an essential role in the development of emotional self-regulation and the stress response (Dawson et al. 1994). Thus, the period of especially rapid frontal lobe growth in human infants—beginning about 6–8 months of age (Chugani 1994)—may be a period of particular vulnerability for infants already at risk for affective disorders by virtue of family history. Future research should address whether experiences during this infancy period have enduring effects on the biochemistry and microarchitecture of the rapidly developing brain, especially the frontal lobe.

Numerous animal studies have documented the effects of maternal experience on the developing fetus. For example, offspring of rhesus monkey mothers exposed to repeated unpredictable stress during pregnancy have been found to be "stress-responsive," exhibiting low birthweights, impaired neuromotor development, attention deficits, and poor exploratory behavior (Schneider 1992*a*, *b*). However, there also has been work in animal models regarding prenatal stress hyporesponsivity (Rosenfeld et al. 1992).

Ultimately, questions remain about whether there is a comparable stress-responsive or hyporesponsive period in humans. How does this stress response vary across age and across species? Is the fetus uniquely protected or uniquely susceptible in some way to certain stressors? For example, the human fetus is protected in many ways against anoxia and hypoxia, requiring much greater oxygen deprivation to create trauma than in a younger or older adult organism. However, it also is true that the fetus is immature and, therefore, it is particularly susceptible at various points in gestation to teratogens, viruses, and other intrauterine influences. Some of these influences might in fact originate from psychosocial stressors that impinge upon the mother. It is important, therefore, to try to delineate those times and those ways in which the fetus is particularly resistant to influences that may stress it and those ways in which it is particularly vulnerable.

Levels of Analysis and Organization

Levels of analysis refers to the conceptual division of a phenomenon in terms of the different classes of questions that can be asked about it; levels of organization reflect the structure at different scales. Cross-disciplinary research on the phenomenon of stress requires continued work collecting data from multiple levels of organization and clarifying the appropriate levels of analysis. These multiple levels not only direct our conceptual models but influence our election of methods, thus limiting or facilitating the scientific work.

Multiple levels of analysis exist within disciplines. For example, in neuroscience, levels of analysis include the course from neuronal and synaptic activity through activation of specific receptors that initiate intracellular processes to resulting synaptic modifications and, ultimately, to changes in behavior of neural networks and intact organisms. Similarly, the study of abnormal behavior and psychopathology includes the levels of individual behavior as they exist within family, school, peer group, work, community, and larger sociocultural contexts. Finally, in order to thoroughly address questions in this research area, the findings from basic molecular, cellular, and systems neuroscience research should influence the design of clinical, intervention (both preventive and treatment), and services research, for example, linking basic psychopharmacology to randomized clinical trials.

Any number of issues involving stress and early development will benefit from addressing critical research questions from multiple levels of analysis. For example, it is apparent that stress-induced hormones derived from the HPA axis do not exert their influences directly, but rather in concert with many other biological events in a variety of different systems. Future research should elucidate the running conversation in the central nervous system among hormones, neurotransmitters, neuromodulators, and other elements of central nervous system (CNS) functions. Prospective research should also consider sleep and sleep regulation as critical aspects of early development and stress, particularly in considerations of arousal in the HPA axis. Early chronic abuse results in the dysregulation of brain stem activity, altering regulatory activities such as catecholamine release, sleep, cardiac reactivity including heart rate and blood pressure, temperature maintenance, and autonomic nervous system startle.

"Allostatic load" is another factor that appears to have importance across levels of analysis. In the developing organism, it is not clear how much and what kind of energy it takes to maintain stability in a changing environment. For example, what is the effect on children's capacities for emotion regulation when they maintain an attachment to their caregiver in the face of physical abuse? Still to be determined are the different constellations of events that should be considered when considering allostatic load, for example, changes in the immune system, cardiovascular system, adrenal function, and behavior.

In a different realm, very little is known about the effects of maternal depression during the prenatal period on fetal development. Multiple factors at various levels potentially could contribute to an unfavorable intrauterine environment, including biochemical changes directly related to the mother's depression and indirectly related to her nutrition, sleep patterns, and stress levels. Future studies in these areas can illuminate our understanding of the experience of stress in the perinatal period and our explorations of the plausible hypothesis that prenatal exposure to stress might predispose an individual to the occurrence of psychopathology by permanently altering neural circuits, possibly by impairing neuronal maturation.

Alternative Pathways

Ordinarily, pathological environments are thought to produce disordered states. While that is one consequence, pathological environments frequently do not produce straightforward pathological outcomes. At the level of the individual and of society, it is known that certain individuals do not seem to suffer poor outcomes, even though they have existed in severely pathological environments. Similar circumstances exist at the cellular and systems levels as well; for example, the ultimate outcome of traumatic brain injury may be without neuropsychological impairment.

To help solve the puzzle of alternative pathways, we need to know how to define those sensitive and critical periods that may influence developmental trajectories and result in the pursuit of alternative pathways to particular outcomes. When is input necessary for basic maintenance; when is specific input necessary; when is the system vulnerable to change or vulnerable to disruption? We also need to know how to measure the construction of these alternative pathways. In order to study changing function in the developing nervous system, for example, electrophysiological methods and imaging methods could be used in a complementary fashion.

Two well-established critical times are the prenatal period (which is extremely difficult to study in humans) and the pubertal period. Two other times that are putative critical periods—in which environmental stimuli may produce irreversible effects or in which a multiplicity of systems may be coordinated—are the perinatal (3 days prenatal through 4 weeks postnatal) and middle childhood (5 years through puberty) periods. For example, during the perinatal period, levels of dehydroepiandrosterone (DHEA) approach adult levels. Infant gonads that have been suppressed by the maternal steroids are suddenly removed from that inhibition and production of steroids surges—males have testosterone levels that are virtually as high as they will be at early puberty, while females have high estrogen and progesterone from their ovaries. Not until the neonate's inhibitory system begins functioning (around 3 to 4 months after birth) do these hormones decrease (McClintock, this volume). McEwen's work suggests that during this age range, the developing nervous system is sensitive to the effects of circulating estrogens, progesterone, and testosterone.

Similarly, the middle childhood period, while described by Freud as a "latent period," actually is quite active with regard to cognitive and social change as well as physiological change, that is, adrenarche. This hormonal change is the same for males and females. So, another potential critical period exists in which the hormones have the capacity to orchestrate many of the other physiological systems. These key ages, beyond the prenatal and pubertal periods, are to be considered as prime times for identifying the reciprocal interactions between genes, the nervous system, hormones, behavior, and the social environment.

Future research should consider how pathological environments influence individuals, and what factors (neurobiological, psychological, or social) within the individuals in these pathological environments actually buffer some of them from morbid psychological and physical outcomes. Specific work should be devoted to understanding the role of trauma at all levels—molecular, physiological, social, environmental—of early development.

Research attention needs be paid to psychosocial as well as biological trauma. These endeavors may be aided by the use of naturally occurring stressors such as labor and delivery, first entry into nursery school, and first entry into elementary school. These stressors should be considered from the perspective of both the child and the parent. Finally, social, political, and economic factors can inform our research. We need to investigate the effects that certain kinds of economic and political events have on the developing organism.

Conclusion

Because stress involves all aspects of human function, it is impossible to understand its nature without studies of the brain. It would be convenient if we could understand the nature of stress and its ultimate effect on behavior and developmental psychopathology without understanding the nature of the brain itself. However, it is impossible to theorize effectively on these matters in the absence of neurobiological underpinnings and constraints.

On the other hand, the possibility that behavior will be an open book once we understand the details of each and every neuron and its development, connectivity, and response properties is likewise misconceived. The details of neuronal development and connectivity cannot, alone, explain behavior and psychology of stress. The participants in this roundtable discussion argued strongly for the benefits of addressing issues of stress and development from multidisciplinary perspectives and from several levels of organization. In addition to committing support for such research, attention and resources may need to be devoted to training a new breed of multidisciplinary scientist in order to accomplish this objective.

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Neuronal Replacement and Segmented Memories

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ABSTRACT

The song system of birds such as the canary and zebra finch consists of 20 or so discrete nuclei and their connections. Some of this circuitry develops late in ontogeny. The study of such a system presents us with the challenge of understanding which part of it does what and when, so that a number of weeks or months later an auditory model has been converted into a vocal copy. Presented with such complexity, one might easily despair. Fortunately, even as we move toward the ambitious goal of understanding the neurobiology of vocal learning, we encounter, here and there, some little paradoxical observations that are, by themselves, interesting. I would like to highlight two, because they are so unexpected and perplexing, and because they force us to think in new ways about how learning happens in the brain.

The first of these paradoxes is the constant replacement of neurons that goes on in the high vocal center (HVC) of adult canaries. HVC is one of the two largest nuclei of the song system. Neuronal replacement there occurs every month of the year, but peaks in late summer and early fall, when canaries acquire a new song repertoire. This observation has lead me to suggest that in some systems memory alters neurons in a permanent manner and that in such systems the whole neuron, rather than its component synapses, is the unit of learning. Such units may have to be replaced to make room for new learning. If true, this is an important insight.

The second paradox I would like to highlight has to do with the duration of memory. Neurons in the caudomedial neostriatum of adult zebra finches respond to complex sounds. This responsiveness decreases as the same sound is presented again and again, and we call this process neuronal "habituation." The duration of this habituation is longest—up to 90

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h—when the stimulus used is conspecific song. Yet this is not a seamless memory. Instead, it is composed of back-to-back shorter memories, each prolongation initiated by a new episode of gene expression and protein synthesis. The number of these episodes, which occur at fixed intervals, hours and days after the end of stimulation, is determined by the nature of the stimulus and its manner of presentation. This is a novel way of thinking about long-term memory.

Both paradoxes—the constant occurrence of neuronal replacement in a nucleus necessary for focal learning and the back-to-back segments that constitute a long-term auditory memory—add fundamental information about things brains do. The discovery of both paradoxes was unexpected and I hope that, in due course, they will help explain how vocal learning comes about. The take-home message is that sometimes you study A and discover B. Perhaps someone else, studying B, will discover A. That is, much of the time, how science works.

The Roundtable Seminar on Cognition

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The Roundtable Seminar on Cognition was one of the plasticity conference's three formal opportunities to discuss the integration of broad domains of scientific investigation. Participants were tasked with synthesizing the discussion of the previous day's symposium on cognition and identifying specific fundamental scientific and clinical problems on which to focus. The panelists (Elizabeth Bates, William Greenough, Douglas Meinecke, Fernando Nottebohm (chair), Pasko Rakic, and Allan Reiss) and the participants recognized the appeal of developmental plasticity as a unifying topic.

Several concepts within the context of cognition seemed promising for a discussion of cross-disciplinary integration. Nottebohm began the session with a presentation of data from his laboratory on vocal learning in song birds. One interesting finding is evidence that new neurons are generated on a seasonal basis in the brains of adult song birds. He suggested adult neurogenesis may be a mechanism to establish new memories in the brains of these animals. As a consequence of this provocative observation, memory became the dominant theme for the panel. Four lines of questions generated meaningful discussion:

- How is plasticity encoded?
- What induces plasticity?
- How much plasticity occurs throughout development?
- Are there special periods in development for plasticity and, if so, can they be exploited for therapeutic value?

Plasticity, in this context, is defined as the ability and degree to which the nervous system can change in response to stimulation. Such change could occur at many levels, from molecular to neural systems. Memory is an example of a cognitive process which is plastic. The topic of how this plasticity is encoded centered on a discussion of where long-term memories reside and how they could be studied. At the molecular and cellular level, the current leading

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mechanism for memory storage in the brain is a form of plasticity known as long-term potentiation (LTP). LTP occurs at the synaptic connections between neurons and involves the strengthening or weakening of a given synapse in response to stimulation (synaptic plasticity).

Research in LTP addresses the molecular events occurring at the synapse, which can be categorized as the biology of excitable membranes or membrane biophysics. For the reductionist, this is often a satisfying enough research question to study, yet it is almost intangible for those seeking to understand memory at the organismal level.

The panel raised the cautionary question of whether the synapse should be casually accepted as the repository of memory. If synapses are the units of memory storage, then they should have the capacity for long-term—across the lifespan—information storage. In the face of such intense study of LTP, it is paradoxical that modern neurobiology has no understanding of the longevity of synaptic states. Therefore, it may not be rational to place too much faith in the synapse as the sole site for the storage of long-term memory.

Given that a single neuron may form thousands of synapses, is it equally appropriate to consider the neuron as the storage site for memory? If the neuron is considered as the repository of memory, then how is memory space allocated in the brain? Two general lines of reasoning were pursued to address this question. First, the hypothesis that the neuron is the unit of memory storage implies that the acquisition of long-term memory involves changes in gene expression which permanently alter the neuron. Second, synaptic states across large numbers of cells and circuits could be sufficient to provide large memory capacity.

A genetic involvement in memory storage implies that protein synthesis should be involved, and this has been tested. Results from experimental animal studies demonstrate that blockade of either gene expression or protein synthesis interferes with the acquisition of new memories. However, the recall mechanisms of the molecular processes that might be involved in accessing genes or proteins encoding long-term memories do not adequately answer some questions. For example, in humans some memories are recallable very quickly, yet others are recallable over variable lengths of time. Why, if genes encode memories, should some memories be fast and others slow?

The panel allowed that a gene-based memory storage model has merit, albeit with limitations, and then questioned whether memory storage capacity has a limit. Panelists familiar with research on human memory suggested that such a constraint may not exist. For example, data on implicit memory for pictures in humans show significant priming of memory as a consequence of a single exposure to a large set of pictures. Recollection up to 1 year later suggests speed, stability, and large capacity.

Similarly, work on human infant memory for the recognition of objects demonstrates that the capacity of the human brain for very long-term memories is inestimable. To account for such a process, Bates reminded the participants that if memory is regarded as a state of some pattern of activation across the staggering numbers of synapses in the brain, then there is ample storage capacity. Moreover, if a very large neural network is in place in which activity patterns are highly distributed, the random loss of synapses would not preclude maintenance or the reestablishment of the original pattern. Citing data from computational analysis of the numbers of synapses on a single neuron and the numbers of neurons in the brain, it was noted that the combinatorial potential for the number of activation states is enormous.

A provocative alternative mechanism to memory storage was offered by Nottebohm, who referred to research on vocal learning in birds to suggest that new neurons could be added to given neural circuits to add new memory (the subject of his opening remarks). Evidence for this hypothesis in other species is lacking, although new neurons are known to be generated in the brains of song birds at times when these animals are in peak learning phases of their lives. A counterpoint to this reasoning is that if the whole neuron were not the unit of learning, and if all learning resided in modifiable synapses, then neuronal replacement would be unnecessary. However, the data for neuronal replacement in birds is clear.

One possibility which could reconcile these views is that different memory mechanisms are exploited in different species. For example, results from cell-birth dating experiments in the rhesus macaque do not support the ongoing generation of significant numbers of new neurons in the adult primate brain. Other evidence that neuronal replacement may not occur in humans is that cognitive abilities rely on stable memory bases. If early learned memories were replaced by new memories associated with the addition of new neurons, then early learned experiences would be lost. Such losses would be crippling to human behavior.

Nevertheless, the possibility of different memory strategies in different species was an interesting concept which stimulated many of those engaged in the discussion. In sum, three models of memory encoding were offered. One model hypothesizes that synapses are established during a developmental period, which subsequently encode events as the variation of the strengths between these connections. A second model allows for active synapse formation and reformation between elements that were not previously connected across the lifespan. Finally, there is the bird song-based neuronal replacement model discussed extensively by Nottebohm.

Greenough commented that the neuronal replacement model is conceptually appealing because it allows for the generation of totally new circuities in the adult brain. Presumably, it does so by actually integrating with information that is already present—new neurons making connections to other new cells as well as older established neurons. The panel agreed that the question of memory encoding merits further evaluation, which could be done using a cross-disciplinary approach.

The exchange of views on memory encoding led to an active discussion on the existence of critical/sensitive periods in development. This concept is often used to explain why some kinds of brain plasticity (recovery of function) occur more readily early in ontogeny than late in life. One example of this phenomenon is the enduring strength of early memories as compared to memories acquired later in life. Specifically, the persistence of accents in the speech of people who have learned a second language after the age of 15 was noted.

Bates cautioned the panel to consider that the evidence on second-language learning suggests a monotonic decrease in the final end-state, but there is no clear cutoff point for this type of learning. She cited a study of language by people many years after they had moved to the United States that found a very long, nonlinear, monotonic decrease in the ability to pick up the fine details of language phenology and morphology. During development, the capacity to learn new language details extends well into the second decade of life.

Rakic referred to his presentation earlier in the meeting regarding the changes in synapse numbers during development in the monkey. Brain synapses are generated in excess early in development in the cerebral cortex and subsequently reduced to adult levels from birth to late adolescence. Furthermore, the changes in numbers of synapses during this period is enormous. Quantitative data on synapses from post mortem brains support an identical process in humans. Therefore, if synapse formation and elimination are involved in learning and memory, then these findings are compelling evidence that childhood represents a particularly sensitive developmental period.

One cautionary note was raised to consider not only that exogenous changes can bring about age-related changes in plasticity, but also that learning itself is shaping the brain. Thus, the ends of critical periods may be the product of learning. Neural network simulations can be used to demonstrate critical period effects with absolutely no exogenous stimuli. Learning itself is, therefore, a causative agent of plasticity. The panel agreed that much more information is needed about the natural history of sensitive periods. This should be followed by a reductionistic search for the factors and mechanisms associated with this process and, importantly, when and if the process ends.

Understanding the neurobiology of sensitive periods may provide the means for correcting developmental errors, inducing brain repair, and devising more effective behavioral interventions. Sensitive periods often have been defined as a time during which a particular circuit change or a particular kind of learning is possible, and the end of the sensitive period occurs when such changes are no longer possible. The panel discussed how this process could be used to advantage in a therapeutic manner. Plasticity that brains normally use for learning may also be a very natural kind of plasticity that could be used for the purpose of brain repair. If it can be determined that developmental events are not temporally restricted, then it may be possible to cause the adult brain to return to earlier processes. One participant was moved to comment that we need to underscore the fact that many human intervention procedures assume that underlying neurobiological changes are being induced, yet the basic mechanisms are unknown. Changes associated with behavioral intervention may be the result of keeping the period of maximal plasticity open longer, or it may actively recruit the formation of new synapses. Clearer answers to these questions would be highly useful in the implementation of clinical treatment of behavioral disorders.

The Cognition Panel explored the representation of speech and language in the human brain at some length as topics for the study of developmental plasticity. The discovery by Broca in the late nineteenth century that lesions of certain brain regions can cause disruptions in language demonstrated that specific cortical regions subserve the functions of speech and language. However, despite numerous articles and texts describing those areas, it is clear that there is not yet a solid understanding of the brain circuits which govern these two related functions. One possible explanation offered for this confusion is that there may not be a defined set of brain regions which process these behaviors—for example, multiple sites interact to produce speech and language. Is it, therefore, necessary or important to search for these circuits? While there was some debate over the answer to this question, some participants stated that scientific rigor demanded that these circuits be defined despite the complexity. Although scientific opinion differed about how this issue should be addressed, it was clear that speech and language are so central to our humanity that this topic is very important for cross-disciplinary research.

Language and speech and the localization of specific functions to certain brain regions introduced the phenomenon of lateralization of function to left or right brain hemispheres. It has been argued that the capture of phonetic encoding and decoding by the left hemisphere is related to that hemisphere's greater ability to process temporally encoded information. One experiment which might reveal the nature of this process is what happens when right and left hemisphere functions are forced to collapse into a single hemisphere. Children who have been treated for intractable epilepsy by removing substantial portions of one hemisphere show remarkable competence on many tasks normally segregated between the two hemispheres. How this occurs is a matter of great basic interest—and may represent the core elements of developmental plasticity at work. However, a price must be paid in exchange for this adaptation to hemispheric removal. Nevertheless, work on laterality may provide important contributions to understanding the basic biology of certain diseases (e.g., dyslexia, the topic of Galaburda's talk in the symposia).

While many behavioral and mental disorders are certainly the result of complex, interconnected etiologies, some disorders are the product of mutations of single genes. Diseases caused by genetic mutations provide powerful models for the study of brain and behavior, as demonstrated by Reiss's chapter on fragile X syndrome. In such disorders, it is possible to link the molecular abnormalities, the physical changes in the brain, and the behavioral disorders with a great degree of confidence. Exploiting these types of disorders to study developmental plasticity should prove fruitful.

Temperament and Plasticity in Childhood

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ABSTRACT

Our current view of temperament in childhood incorporates aspects of both stability and developmental change. Initial differences in emotional, attentional, and motor reactivity are seen as increasingly modulated by the development of behavioral inhibition and voluntary attentional systems. In research on temperament, we are now finding increasing agreement that a small set of dimensions can serve as a starting place for the study of this development. These include individual differences in fear, irritability, positive affect and approach, activity level, orienting, and effortful control. One of the current challenges for our field is to identify measures to allow the study of individual differences in temperament as they develop.

One approach we have found helpful in the study of attention is considering multiple levels of analysis. Thus, we can study orienting as behavior, using measures of direction and duration of fixation from videotape records. Second, we can study orienting as involving a set of mental operations such as obligatory looking or inhibition of return. Third, we can study orienting as a neural system. We use behavioral marker tasks that have been linked to neural function to assess development of orienting and volitional systems. Finally, we can study cultural and experiential influences on orienting, as children learn locations in their environment that are important to monitor. Study of each of these levels of analysis informs our research and understanding of the other levels.

In this introduction to our roundtable discussion, two examples of the multiple level approach from our laboratory are presented. The possibility that neurological development may be reflected in children's behavioral propensities is also considered. Finally, research by others on temperamental contributions to the development of attachment and conscience,

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stressing multiple pathways to developmental outcomes and their relation to issues of plasticity, are reviewed.

Several major themes of our conference may be used to introduce this roundtable discussion. First, although we approach developmental processes from diverse viewpoints and training, we are engaged in the study of conserved systems in evolution, especially self-regulative or self-organizing systems with implications for plasticity and change. Second, because these systems develop and are multiply influenced, their adequate study requires longitudinal and experimental study at multiple levels of analysis. Third, a critical aspect of our analysis is behavioral. Behavioral study is needed not only to establish meaningful links with the neural substrate, but to permit study of the organism in context, allowing examination of influence from the social and individual levels down as well as from neural levels up. Finally, interactions occurring across these levels bring us back to the first evolutionary issue: social behavior is of critical importance to survival. It is a requisite to mate selection, care of the young, and mutual protection, to name only a few. I touch upon several of these issues in relation to our work at Oregon on the development of temperament, with an emphasis on its attentional aspects.

In our research and thinking on temperament and attention, we have noted that plasticity involves not only the molecular, synaptic, or systems-level changes related to experience and maturation, but also attentional and affective-motivational processes that organize behavior and thought from moment to moment (Rothbart, Posner, et al. 1994). These systems allow for the relatively immediate organization of behavior and thought in response to challenge and in the pursuit of long-term goals. In addition, attention and motivation support practicing and mental problem solving, thereby affecting long-term structural changes. Indeed, some kinds of learning occur only when attention is directed to the task performed (Curran and Keele 1993).

The systems supporting attention, affect, and motivation themselves develop, and organisms vary in the strength and flexibility of deployment of these systems. In temperament, we study individual differences in emotional and motor reactivity and attentional self-regulation. A graphic example of temperament is seen in the work of Shirley (1933).

Shirley was an outstanding observer of child development, and her descriptions of the prone motor progression can be seen in developmental texts today. When she set out to intensively study a group of infants over a 2-year period, her initial focus was on motor development. She was so struck by the children's variation in what she called the "nucleus of personality," however, that she added a third volume reporting individual differences among the infants (Shirley 1933). She describes how the two children each experiences the "same" situation but in quite different ways; each is practices a different set of behaviors; each creates a different social relationship with people in the environment. Escalona (1968) noted that the "effective environment" operates through the filter of the individual child's nervous system, creating a whole set of variations on the "objective environment." In turn, individual children create different environments for their caregivers (Bell 1974).

This chapter first reviews recent advances in our understanding of the structure of temperament, indicating a closer connection with conserved systems in evolution than we had originally thought. Second, approaches to the study of temperament from our laboratory demonstrating a multilevel approach are described, with suggestions for some institutional arrangements that might support collaborative research.

Dimensions of Temperament

Investigators' choice of dimensions of temperamental variability has been strongly influenced by the nine dimensions put forward by Thomas and Chess' (1977) New York Longitudinal Study (NYLS). Based on a content analysis of interviews with parents of young infants, the NYLS yielded temperamental dimensions of approach-withdrawal, mood, threshold, intensity, activity level, rhythmicity, adaptability, distractibility, and attention span-persistence. Because the NYLS was particularly focused on clinical issues, however, no attempt was made to develop homogeneous and relatively independent measures based on these constructs.

This led to problems in subsequent research in that measures of some of the NYLS-based constructs were so highly intercorrelated that they might have been measuring the same construct. In addition, for constructs such as intensity and threshold, it proved extremely difficult to develop homogeneous measures. These problems led researchers to carry out item-level factor analyses of data based on the NYLS constructs (Hagekull and Bohlin 1981; Sanson et al. 1987), and this research in turn led to an important set of revisions of the NYLS list of constructs (reviewed by Rothbart and Bates 1998).

This revised list, like the NYLS list that preceded it, describes individual differences in temperamental dispositions, as reflected in the latency, intensity, and duration of emotional and motor activity and in the deployment and control of attention. For the period of infancy, the revised list of temperament constructs included infants' proneness to fear or behavioral inhibition, irritable distress (anger/frustration), approach and positive affect, activity level, persistence, and (in some instances) rhythmicity (Rothbart and Mauro 1990).

In later childhood, the list included fearful distress and behavioral inhibition or shyness, irritable distress, positive affect and activity level (approach/extraversion), effortful control/task persistence, and (in some instances) agreeableness/adaptability (Rothbart and Bates 1998). Significant but modest stability of these characteristics has now been reported over relatively long periods, including measures taken in infancy to middle childhood and the preschool to teenage years (see review in Rothbart and Bates 1998). However, because the magnitude of stability coefficients ranges from small to moderate, considerable change in the rank order of individuals on temperament measures occurs, indicating the likely influence of other social and individual developmental factors.

In these revisions of the NYLS dimensions, the affective-motivational systems of fear, frustration, and approach were identified. These dimensions in turn demonstrate some conceptual overlap with personality researchers' Big Five personality superfactors (Digman and Inouye 1986; Goldberg 1993). The Big Five or Five Factor Model for personality includes extraversion (similar to approach and positive affect in infancy and childhood), neuroticism (similar to fear), conscientiousness (similar to effortful control), and agreeableness (with an opposite pole similar to irritability or anger). The Big Five construct of openness to date shows little conceptual similarity to the temperament dimensions.

There is also similarity between the temperament constructs and those of neuroscience approaches to emotion and attention in adult subjects and across species (Gray 1991; LeDoux 1987, 1989; Panksepp 1982; Posner and Peterson 1990; Zuckerman 1995; and others). Dimensions of temperament, including attention, have also been linked to neural circuitry and neurotransmitter function (see reviews by Rothbart, Derryberry, et al. 1994; Zuckerman 1995), and the emotional, arousal, and attentional aspects of temperament appear to constitute important conserved systems in evolution.

Development of Attention

Because attentional systems have been identified with an underlying neural circuitry, because they develop, and because they serve important self-organizing functions in the service of developmental plasticity, we have been especially interested in their development, with our current research extending over the first 3 years of life. We have studied attention by employing marker tasks identified in research on adults and brain-lessioned patients.

Recent studies using neuroimaging techniques have illuminated our understanding of the neural systems activated in human orienting to sensory events and in the executive control of action and the expression of emotion (Posner and Richly 1994). These imaging results are congruent with results of brain lesion work and studies of normal adult subjects performing simple cognitive tasks. We have used simple model tasks that are related to specific neural activation to trace the development of orienting and effortful control in infants and young children. In visual orienting, we have identified two major periods of early development: the first between 3 and 6 months of age, the second late in the second year of life. I focus here on the first period.

During the period of 3–6 months, the infant develops the ability to disengage attention from a visual stimulus in order to move it to a new location and to anticipate the occurrence of a predictable location based upon central cues (Johnson et al. 1991). This development is likely dependent on maturation of brain networks involving parietal, thalamic, and midbrain mechanisms for shifting attention (Posner and Petersen 1990). Later in the second year, the child develops the ability to more flexibly shift the locus of attention depending on the context of stimulus presentation (Ruff and Rothbart 1996). We have hypothesized that the 18-month shift requires development of frontal structures underlying more general attentional activity.

In applying marker tasks to populations of infants and young children, we have studied a number of aspects of an orienting system. One task assesses Inhibition of Return (IOR), the tendency not to return (over a period of 2-3 sec) to a location previously attended either overtly by eye movements or covertly by shifts of attention. The task involves presentation of a stimulus to the right or left of fixation while the infant is focused on a central location. After the infant's eyes or attention have moved to this location, the stimulus is removed from the screen, the infant is drawn to the center, and identical stimuli are presented on both sides of fixation. IOR is shown when infants demonstrate a preference for the location not previously visited.

Development of IOR shows a U-shaped function. Newborns demonstrate IOR (Valenza et al. 1994), but 2- and 3-month-olds do not (Clohessy et al. 1991). IOR increases from 3 to 6 months of age, along with infants' ability to program a single eye movement to a stimulus. After this time, it remains at close to adult levels. These results suggest that basic computations for IOR are present at birth but are implemented only when a stimulus has evoked a ballistic eye movement, either reflexively or as a function of orienting experience (Posner et al. 1997).

The 2- to 3-month-old also shows difficulties in disengagement of attention from a central stimulus, but by the age of 4 months, infants come to easily disengage from a location even when the stimulus remains present (Johnson et al. 1991). Development of this "disengage" operation in infants' performance seems to fit well with maturation of parietal areas (Chugani et al. 1987) in brain-imaging studies. In addition, infants' difficulty in disengaging prior to about 4 months is congruent with behavioral data on "obligatory looking." These observations have been reported for some time in the developmental literature and were reviewed by us early in our work (Posner and Rothbart 1981). In obligatory attention, infants remain fixated on one location for extended periods, not moving the eyes even when the infant appears to have become distressed by the stimulation in that location.

At a social level of analysis, the period of 2-3 months also corresponds to the time of frequent vis-a-vis interactions between caregiver and child, when the mother also reports that her child now seems to be an individual, that the infant seems to be showing love for the parent, and that she has developed genuine feelings of love toward the infant (Robson and Moss 1970).

By 4 months of age, however, infants seem more interested in other events in the environment, causing difficulties for parents who might wish that their infant would maintain this close interaction. At this time, in our culture, the parent often faces the child in an outward direction rather than persisting in the vis-a-vis position (see review in Johnson et al. 1991). Putting these social observations together with the laboratory findings, we see that important developmental events are occurring at the neural, behavioral, cognitive, and social level of analysis.

Over the period of 3 to 6 months, infants also develop the ability to predict the location of one event based on a central cue and to learn simple sequences of locations (Haith et al. 1988; review by Ruff and Rothbart 1996). This flexibility of attention may also be important in moderating the infant's expression of distress. Over the early months of life, parents increasingly use distraction to soothe their infants, even though in our research the soothing tends not to persist after the distracting stimulus is removed. We have found evidence for a "distress keeper" that appears to hold an affective computation over a period of distraction by an external stimulus when the child is not expressing the emotion. A return to previous distress levels occurs even when the distracting stimulus has been presented to 3- and 6-month-olds for periods up to 1 min (Harman et al. 1997).

We are currently studying later developments of executive attention systems associated with effortful control in the second to third year of life. These systems likely involve frontal structures and may be related to the development of conscious control over thought and action (Posner and Rothbart 1991; Rothbart et al. 1990). Executive control allows inhibition of a dominant response in order to perform a subdominant response. The classic task demonstrating this capacity is the Stroop task, which requires a subject to name the color of ink in which words are printed. When the word prints the name of another color, for instance, the word red is printed in blue ink, the subject must inhibit the tendency to read aloud the word red and instead give the ink color name. Efficient performance on Stroop and Stroop-like tasks depend upon resistance to interference and are associated with frontal activation (see review by Posner and Raichle 1994).

Gerardi (Gerardi et al. 1996) in our laboratory has now developed a spatial Stroop-like task that can be used with children as young as 24 months that may prove to be a marker for development of frontal brain regions linked to effortful control. In this task, children match one of two buttons covered by animal pictures with the picture of an animal presented on a computer screen. The animal on the screen is presented either on the same side (spatially compatible) or on the opposite side (spatially incompatible) of the correct button. Overall, toddlers from 24 to 36 months are more accurate and respond more quickly to compatible than incompatible trials. Their accuracy also improves considerably with age across the 24- to 30- to 36-month-old periods we have assessed.

Moreover, children's performance (their accuracy and interference in the incompatible task as measured in reaction time) is related to their mothers' reports of the children's temperament, using the Children's Behavior Questionnaire (Rothbart, Ahadi, et al. 1994). Children who were more accurate and/or less slowed by the conflict were reported by their mothers as showing higher attentional focusing, higher inhibitory control, and lower impulsivity. In addition, anger/frustration scores were significantly lower for the older children who had shown greater accuracy on the task.

These findings are exciting because again they relate to cognitive, neural, and behavioral levels of analysis. They also have important implications for children's socialization, as indicated in the work of Kochanska on moral development (e.g., Kochanska 1995). Because the frontal development that is likely related to development of this capacity occurs over an extended period (Huttenlocher 1979), it will also be important to explore possible plasticity of the executive system during its period of development.

In our work at Oregon, we have found it possible to achieve multiple-level analysis chiefly by collaboration among investigators with different but complementary areas of expertise. Although each investigator must relinquish some control in this endeavor, multilevel research has proved fruitful for us. We have also attempted to encourage research that cuts across traditional areas by encouraging doctoral students to carry out empirical studies related to problem rather than area (e.g., developmental, cognitive, neuroscience). Students' research committees include faculty with expertise at levels of analysis needed to address the research problem or question. Our work thus suggests that the development of attentional aspects of temperament is a problem amenable to research at multiple levels.

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Summary of the Social Behavior Roundtable Discussion

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It is imperative that we understand social behavior as comprehensively as possible. The roundtable discussion indicated that the biomedical implications of this area of study are very compelling, yet barriers exist to the translation of basic social behavior research into reliable health applications. One important barrier is the availability of relatively few unifying principles. This may be due in part to the sheer complexity of the phenomena that encompass the development of social behavior. The discussion addressed this issue by covering a number of interesting topics which reflected this complexity: (1) the usefulness of animal models for developing viable research strategies, (2) social regulation of emotions, (3) studies of transgenerational effects on individual development, (4) the role of cognition in shaping social development, and (5) differences in methodology for different levels of organization.

The usefulness of animals to better understand human social behavior was raised initially as part of the formal presentations, then later in the roundtable discussions. Social behaviors include important functional components which are certainly aspects of conserved systems in evolution. This was discussed in terms of the hormone, oxytocin, contributing to the formation of strong attachments and mental preoccupations with specific individuals as well as in studies related to strongly self-organizing or self-regulating systems such as the development of attention in mother-child interactions. Such similarities among species can be very provocative and could generate interesting new research.

However, differences among species also need to be addressed. For example, significant differences in reward systems which may exist between nonhuman primates and rodents can produce important and interesting comparisons for the study of affective systems. The discovery of common principles in different species is promoted by the evolutionary/comparative perspective, which encourages examination of the same phenomena at whatever level of analysis may be of interest.

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There was considerable discussion of phenomena related to the transgenerational transmission of maternal behavioral patterns and its effects on offspring behavioral development. This set of phenomena was also referred to as "nongenomic" inheritance of behavioral traits to distinguish it from genomic inheritance. Even at the molecular level, nongenomic mechanisms may play a role in transgenerational continuities. Changing social interactions at different stages of development are capable of altering the expression of genes in the nervous system, which may lead to altered responsiveness to environmental stimuli.

At the same time, genes can overexpress their products in the context of broader neural and behavioral systems and lead to altered brain function and social behaviors. Research with knockout and transgenic mice will inform us about some aspects of the organization of social behavior. For example, we need a better understanding of the relationship between a social behavior and its pathology—are attentional deficits a product of the same system that regulates the development of normal attentional abilities? In the near future, gene-targeted animals may shed light on the continuity or distinctiveness of normal from pathophysiological functions.

In humans, data on nongenomic transmission of behavioral patterns across generations is obtained through costly longitudinal studies. But this process can also be studied more mechanistically in animals. Egg-laying vertebrates provide a particularly interesting model: mothers transfer or deposit hormones into the egg yolk, and these hormones have significant long-lasting consequences on the development of the hatchlings into adulthood. For example, canaries that have been exposed to a high physiological dose of maternal testosterone in the egg are more aggressive as adults. In mammals, individuals with high levels of vasopressin, for example, might show more territorial behavior. Such individuals would be less likely to go into a communal social setting, and this would alter the social environment in which their infants were reared, changing the infants' hormonal status.

Environmental contributions to nongenomic transmission of behaviors across generations (poverty, substance abuse, abusive parents) may themselves depend on modified central nervous system responses, including structural alterations of the brain. A negative cycle of nongenomic transmission of continued impulsivity and aggression across generations may be mediated through the enduring effects of childhood abuse. Understanding the connections between brain development at the cellular level and the emergence of behavioral capabilities can provide a medical substrate for breaking this negative cycle.

Pair-bonding and affiliation were presented as compelling illustrations of how connections between emotions and social behavior can help us understand the physiological basis of vulnerability to certain forms of psychopathology. Indeed, a major theme which emerged from the conference was that social behavior and social systems are powerful regulators of emotions and may be unique windows for examining affective disorders. In addition to positive emotions associated with attachment, pair-bonding is also a window for understanding fear and stress reactivity, since for most mammals, attachment responses must overcome the individual perceptions of danger associated with the approach of others.

Parental behavior is an excellent experimental substrate for examining the design principles of genomic and neuroendocrine regulatory systems. The development of early infant behavior and physiology is influenced by several classes of functionally overlapping events: sensorimotor experience—thermal, visual, tactile, and olfactory inputs; and need states—homeostasis, rewards, and motivational states regulated by nutrient ingestion. In early development, these events are regulated by social processes, like parenting. Parenting is not only a matter for survival of the infants, but it also influences developmental rates and trajectories. Early abrupt separations from parents can lead to abnormal response patterns and pathophysiology caused by the sudden withdrawal of discrete regulatory processes.

Some social environments have been identified as critical for the development of cognitive abilities. Conversely, it was understood that the course of cognitive development is critical to the formation of attachments. One system to study the interactions between cognition and social development which was presented in the roundtable discussion was the phenomenon of "obligatory attention." About the time that the young infant is showing this obligatory attention, changes in the infant's perceptual apparatus occur that allow for scanning within the set of facial features. This is just the moment (around 4 months of age) when mothers are reporting feelings of love for their babies. The mother's face is critical; gazing at a checkerboard pattern at this time, rather than at the mother, produces a distress response.

The regulation of stress responses by gaze and physical contact is relevant to obligatory attention. If, as a result of early stressful experiences, cortisol levels become increased in the hypothalamus, animals will misperceive risk or danger, misinterpret threat, remain hypervigilant, and engage in more frequent fighting. Early stressors alter neurotransmitter systems, such as the underproduction of serotonin and the inhibition of vasopressin neurons which control fighting. Such research at the boundaries between cognitive and emotional development illustrate the need for a broad integrative perspective in the analysis of social behavior and the impact of early experiences.

In the discussion, emphasis was placed on the need to study the role of sensitive transitional periods. A focus on periods of heightened sensitivity to particular inputs will facilitate linkages between studies of brain plasticity and the emergence of new patterns of behavior. Periods of developmental transition such as the onset of walking or multiword speech, are important and complex. Although such transitions are typically associated with needed emotional adjustments, they are not equivalent in the way neural and behavioral systems interact. Similarly, gene-environment interactions will affect behavior in unique ways depending on the contexts provided by the developmental stages and particular systems under consideration.

The environment has a powerful influence on brain development and on the associated development of social functioning in children. Although animal studies have provided some insights, the nature of this influence in humans, including the physiological and behavioral underpinnings, is poorly understood. The emergence of emotional expression in children is very dependent on social input, and dysfunctional environments produce serious social impairments in children. Among the most detrimental experiences are those which exert their influence early and often: repeated exposure to traumatic events, fetal exposure to alcohol and drugs of abuse, and chronic exposure to lead and other heavy metals. Social behavior was recognized as an important marker for the adequacy of environmental support systems that facilitate successful growth and maturation. Emotional systems can play an important role in facilitating or hindering learning, as well as in the organism's interactions with the environment more generally.

One important part of the roundtable discussion addressed scientific barriers to multidisciplinary interactions. Methodological issues sometimes interfere with the development of unifying concepts across levels of analysis. Simple, linear, cause and effect explanatory models do not easily apply to human longitudinal studies. Difficult issues associated with the methodology of social analysis need to be more broadly recognized and understood by investigators working at other levels. A very important marriage needs to be established between demography, population demography, and methodology to characterize where populations reside at any given time. This kind of information would be the basis for countering a "casualness" that exists in some groups related to sampling methodology, how mechanisms are inferred, and how findings become generalized. Advancements of this methodology can help address a critical need to describe the ontogeny of social systems that conceptually integrates concepts like attachment, peer relations, peer status, and maternal behavior.

In addition to methodological considerations, a proposal to integrate the research agendas concerned with social structures with the individual/biological level would require increased attention to social analysis beyond the dyad. An example of a longitudinal study in North Carolina was cited as an exemplary combination of methods that determined mechanisms and at the same time characterized the population to which those inferred mechanisms applied.

The physical and psychological environment for a particular species can be seen as an adaptation to ensure the safety, normal growth, and development of the offspring. Cognitive and emotional systems serve to support the social interactions. Such preconditions are necessary to permit an infant to thrive by assuring, for example, normal patterns of sleep and wakefulness, by establishing appropriate systems of reward and motivation, and by setting the stage for mobility and communication.

Poster Presentations

Long-Term Developmental Consequences of Repetitive Pain in Neonatal Rat Pups

K.J.S. Anand and P. M. Plotsky

Long-term behavioral changes have been noted during childhood in ex-preterm neonates. These may result from repetitive pain that occurs during routine neonatal intensive care.

Method. We stimulated the paws of newborn rat pups with either needle prick (pain, P groups) or cotton tip (tactile, T groups) from 0 to 7 days after birth. Rats were stimulated either once (P_1, T_1), twice (P_2, T_2), or four times (P_4, T_4) a day at hourly intervals. All rat pups were reared under identical conditions on days 7–22, weaned on day 23, and then housed in sex-matched groups. Effects of early pain on weight gain and pain threshold during development were investigated by body weights measured on days 8 (P8), 15 (P15), and 21 (P21) and pain threshold measured on days 16 (P16), 22 (P22), and 24 (P24) using the modified and classical hot plate (HP) tests. After 60 days, adult rat behavior was investigated by the alcohol preference test, defensive withdrawal test, social discrimination test, air-puff startle, and the hot plate test. ACTH and corticosterone levels were measured at baseline and 3, 6, 9, 12, 18, and 30

Groups	Weight on P8	Weight on P15	Weight on P21	Modified HP on P16	Modified HP on P22	Classical HP on P24
P_1 (N=13)	17.4±1.2	32.3±1.5	50.5±3.5	5.9±1.2	3.3±0.7	13.2±3.8
T_1 (N=11)	17.6±1.9	32.7±1.1	50.5±2.4	5.6±1.0	3.6±0.9	13.6±3.4
P_2 (N=12)	15.3±1.5*	30.0±2.1**	45.9±2.8**	6.0±1.7	3.6±0.7*	13.8±4.0
T_2 (N=12)	16.4±1.1*	32.8±1.9**	51.3±4.9**	6.1±1.0	4.3±0.9*	13.9±4.7
P_4 (N=12)	18.3±2.0	35.3±2.5	55.2±4.5	5.0±1.0*	3.9±0.5**	12.1±4.1#
T_4 (N=11)	18.5±1.1	34.1±1.4	54.4±3.6	6.2±1.4*	5.5±1.6**	15.1±4.5

Weight measured in grams; HP threshold measured in seconds; all values—Mean±SD
* p<0.05, ** p<0.005, # p=0.1.

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minutes following air-puff startle. Results were compared between the pain and tactile groups by ANOVA and t tests.

Results. Differences in weight gain (groups P_2 vs. T_2), and pain threshold (groups P_2 vs. T_2; P_4 vs. T_4) in preweanling rat pups imply that repetitive pain during the neonatal period may have long-term developmental effects. No differences occurred in adult rat behavior between the P_1 vs. T_1 or P_2 vs. T_2 groups. Rats in the P_4 group showed a greater preference for alcohol (55±18 percent vs. 32±21 percent; $p=0.004$, t test), increased latency in exploratory and defensive withdrawal behavior ($p<0.05$); and a prolonged chemosensory memory in the social discrimination test ($p<0.05$). No significant differences occurred in corticosterone and ACTH levels following air-puff startle or in the hot plate pain thresholds between the P_4 and T_4 groups.

Conclusions. Repetitive neonatal pain causes decreased weight gain and pain thresholds during development, leading to stress vulnerability and anxiety states in adult rats. Changes in brain development may lead to such behavioral differences, also observed in preterm neonates exposed to neonatal intensive care.

Elimination of Dopamine Receptors in the Prefrontal Cortex and Striatum, but not in the Nucleus Accumbens During Periadolescence: Evidence for Regional Differences in Developmental Plasticity

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The brain demonstrates remarkable plasticity during development, remodeling itself to attain its adult configuration. Much plasticity is related to the overproduction of synaptic connections and receptors during childhood. However, following puberty, the prominent elimination of synapses and receptors may serve as the basis for waning plasticity with age. The fundamental processes of receptor overproduction and elimination may have direct relevance to the time course and emergence of a number of psychiatric disorders. The purpose of the present study was to ascertain whether dopamine receptor overproduction and elimination occurred at the same time, and to the same extent, in the cortex, limbic system, and striatum.

The density of D_1 receptors (labeled with ^{3}H-SCH-23390) and D_2 receptors (^{3}H-YM-09151-2) was examined with quantitative autoradiography in male rats at 25, 35, 40, 60, 80, 100, and 120 days of age (6 subjects per cell). We found that D_1 and D_2 receptor density (fmol/mg protein) reached a maximum at 40 days of age (the periadolescent period in the rat) in the striatum, prefrontal cortex, and nucleus accumbens. In the striatum, D_1 and D_2 receptor density decreased by at least 35 percent by 60 days and remained attenuated at 120 days of age. Receptor pruning in the prefrontal cortex was similar to that observed in the striatum, except that the pattern was more protracted and dramatic. For example, at 120 days the relative density of D_1 receptors declined 62.7±27.3 percent more in prefrontal cortex than striatum ($t_{10}=2.84$, $p=0.018$). The nadir was reached at 80 days in striatum, but delayed until 100 days in prefrontal cortex. Similarly, the relative D_2 receptor density nadir occurred at 60 days in striatum and 100 days in prefrontal cortex. In contrast to the marked receptor loss in striatum and prefrontal cortex, D_1 and D_2 receptor density in the nucleus accumbens was the same at 100 and 120 days as at 40 days. These findings suggest that limbic and nonlimbic areas follow different patterns of plasticity across the periadolescent period.

We propose that overproduction of receptors and their elimination occurs as

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a tradeoff between acquisition of new skills and processing speed. As the brain matures, these connections are refined to reach their adult configuration, and aberrancies in this process may have important ramifications for understanding the emergence of psychiatric disorders. Pruning of dopamine receptors occurs at the earliest age in the corpus striatum, possibly related to relatively early development of motor patterns. Decreased receptor density in this region may have some relation to the waning of hyperactive symptoms in ADHD and motor tics in Tourette's disorder, which often occurs after periadolescence. The delayed loss of D_1 and D_2 receptors in the prefrontal cortex may have important implications for the emergence of schizophrenia in late adolescence and early adulthood. In contrast, the lack of pruning in the nucleus accumbens may reflect the importance of being able to form new affective associations throughout life. Pruning of D_1 and D_2 receptors in female brains may explain gender differences observed in these disorders. Studies are currently underway to compare the pattern of pruning between males and females across the periadolescent period to determine if the female brain undergoes such rapid and dramatic loss in receptors.

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Individual Differences in Recognition and Recall Among 9-Month-Old Infants: Possible Implications for Research on Neural and Functional Plasticity

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One of the important functions of infancy and early childhood is the development of the ability to represent and recall events from the past. Until recently, this developmental milestone has been thought to emerge in conjunction with the onset of Piaget's sixth substage of the sensorimotor period (approximately 18–24 months of age). This assumption has now been challenged by evidence of the ability of young infants to recall events after very long delays. Although it has become clear that the ability to represent and recall information emerges earlier than previously thought, the question of whether there are individual differences in this behavior is only beginning to be explored. Individual differences, if present, may be indicative of plasticity in the development of a long-term memory system. To address these issues, recall memory was examined in 9-month-olds. Thirty infants were tested in a long-term memory study using measures of elicited imitation. In this paradigm, infants are shown a novel event and are allowed to imitate the event after a delay has been imposed (1 month in the current study). In addition to this nonverbal analog to verbal recall, recognition memory was measured using event-related potentials (ERPs). Infants were exposed to three events during each of three exposure sessions separated by 24 to 72 hours. Recognition memory was tested using ERPs after a delay of 1 week. Recall memory was tested 1 month following the ERP session. Overall, infants successfully completed more of the events to which they had previously been exposed than new events. The results also suggested individual differences in memory ability. About 50 percent of 9-month-olds recalled the events after the 1-month delay, which replicates previous results. Individual differences in recall were predicted by individual differences in recognition memory as evidenced by neurophysiological measures 1 month prior to recall testing. Although it appeared that all infants treated familiar events differently than novel events (i.e., ERP responses for both groups differentiated familiar from novel events), there were differences in the topography of the ERPs between groups, suggesting differences in the neural substrate underlying performance. Correspondence between individual differences in brain activity and behavior may provide a useful tool in future investigations of individual differences among infants from differing experiential backgrounds.

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Evidence That Activity-Dependent Anterograde Secretion of BDNF Affects the Development and Survival of Target Neurons in the Central Nervous System

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J. Maclean, and F. Miller

We have hypothesized that early exposure to an adverse environment leads to permanent neural alterations, which in turn leads to development of behavioral abnormalities. To test this hypothesis, we have focused on the central noradrenergic system, which broadly innervates structures in the hypothalamic-limbic axis and which has been implicated in the genesis of a number of behavioral abnormalities. We have generated a transgenic mouse (DBH: BDNF) that overexpresses brain-derived neurotrophic factor (BDNF), a member of the NGF family of growth factors, in noradrenergic and adrenergic neurons. Our studies with these mice indicate that BDNF plays an unexpected, nontraditional role in the central nervous system. BDNF appears to be anterogradely trafficked into axons and nerve terminals and secreted onto target neurons in an activity-dependent fashion. Specifically, in wildtype and transgenic animals, we show that BDNF is present in axons throughout the brain, with punctate staining around neuronal cell bodies reminiscent of synaptic terminals.

We next examined one target of noradrenergic neurons, the cortex, to determine whether target neurons "saw" increased BDNF in the DBH:BDNF mice. Assays of the TrkB/BDNF receptor demonstrated that, although TrkB levels were not altered, the baseline level of TrkB activation was increased in transgenic versus control mice. Moreover, pharmacological activation of noradrenergic neurons led to an increase in TrkB activation within 30 minutes in the cortex of both control and transgenic mice. To determine whether increased BDNF from noradrenergic axons in the DBH:BDNF mice affected cortical structure, we used a transgenic mouse that expresses a panneuronal marker gene (Tal:nlacZ). In Tal:nlacZ–DBH:BDNF crosses, cortical structure was perturbed. Finally, to determine whether anterogradely secreted BDNF could affect neuronal survival, we focused on facial motoneurons, which receive abundant noradrenergic innervation. In the DBH:BDNF mice, axotomized facial motoneurons were rescued from cell death by the increased noradrenergic BDNF. In summary, these data support the following conclusions: (i) BDNF is secreted out of axons onto target neurons, (ii) increased

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BDNF secretion results in TrkB activation on target neurons, (iii) increased secretion of BDNF into the cortex during development leads to alterations in cortical organization, and (iv) increased secretion of BDNF onto developing target neurons can modify neuronal survival. Together, these data support the hypothesis that activity-dependent release of anterograde BDNF may provide a molecular mechanism for altering neuronal development as a function of neural activity.

Prenatal Stress Alters Social and Adaptive Behavior in Adolescent Rhesus Monkeys

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Previous data from rodent studies have indicated that stress experienced by mothers while pregnant may result in behavioral and biological abnormalities in their offspring. In humans, prenatal stress has been associated with increased risk for childhood psychopathology in the offspring of mothers reporting stress during pregnancy. We have previously reported that offspring of monkey mothers exposed to noise stress during mid-to-late gestation showed lower birth weights, impaired neuromotor development, attentional and cognitive deficits, and more disturbance behavior under stress as infants. Followup studies of the monkeys as juveniles showed that the prenatally stressed group showed enhanced behavioral response to stress, enhanced ACTH response to stress, and heightened norepinephrine response to stress.

Here we present evidence for the long-lasting effects of prenatal stress on social and adaptive behavior in adolescent rhesus monkeys. Seven prenatally stressed (PNS) monkeys and seven control monkeys from undisturbed pregnancies were tested under mildly challenging conditions at approximately 4 years of age. When observed during a 3-week period following separation from peer cagemates and new group formation, PNS monkeys showed more locomotion, stereotyped behavior, self-clasping, and general disturbance behavior than controls. Social behavior was also affected by prenatal stress treatment. Control animals showed approximately six times more play than PNS animals. PNS males showed the greatest amount of clinging to other animals and the largest increase in contact with other animals over the period. Group differences were also found when the monkeys were observed as cagemate groups or alone in a large playroom (open field) environment. Control monkeys showed more exploratory behavior and a greater increase in exploratory behavior over time than PNS monkeys. Durations of inactivity (interpreted as freezing) were greater in the PNS monkeys than controls. Control animals showed a decrease in vocalization (an index of distress) over time in the playroom, whereas PNS animals showed the opposite pattern. In the group-testing condition, control animals spent more time in proximity to and contact with cagemates than PNS animals and showed a greater increase in proximity over time than PNS animals.

These results indicate that prenatal stress can have long-term effects on

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behavioral regulation in rhesus monkeys that persist into the adolescent period and may have relevance to some populations of children with psychosocial difficulties. For example, the behavioral characteristics of the PNS monkeys resemble those of children described as temperamentally inhibited. Like inhibited children, the PNS monkeys showed longer periods of inactivity; engaged in less exploratory behavior, less play, and other social behavior; and showed more evidence of disturbance under conditions of novelty than controls. Consistent with the human data showing stability in maladaptive temperamental traits throughout childhood, maladaptive temperamental traits identified in infancy in the PNS sample continued to be manifested in the adolescent period. Given that temperamental characteristics of children are believed to have a constitutional basis, it is possible that prenatal stress may be an important predisposing factor for an inhibited or difficult temperament in both human and monkey infants.

The Neuroecology of Memory in Food-Storing Birds: Predispositions and Plasticity

N.S. Clayton

Food-storing birds have evolved a remarkable feat of memory. Having hidden hundreds to thousands of food caches, each of which is typically hidden in a separate site and scattered throughout their territory, these birds use memory to retrieve their caches when they return hours to months later. In laboratory tests, food-storing members of the Corvidae (crows, magpies, jays, and nutcrackers) and Paridae (titmice and chickadees) have a better spatial memory or rely more heavily on spatial cues than do their nonstoring counterparts. In terms of the brain, the hippocampal formation is crucial for successful retrieval of stored food, and this structure is larger in species that store food than in those that do not. Studies of the dual ontogeny of behavior and brain show that the hippocampal formation is remarkably plastic. Rates of hippocampal cell birth and cell death can be influenced by experience of food-storing and associated memory-based retrieval. These findings have led to the development of the food-storing system as a model for investigating fundamental questions about the relationship between hippocampus and spatial memory in a naturalistic environment.

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A Sequential Multimodal Approach to the Supplemental Stimulation of Premature Infants: A Treatment Model and Preliminary Findings

J.N.I. Dieter

In an effort to decrease the immediate adversities and later developmental deficits associated with premature birth, numerous researchers have examined the effects of various types of supplemental stimulation on the behavioral organization, growth, and neurobehavioral maturation of premature infants. While such research has shown positive effects, the field has been criticized for its lack of both theoretical direction and sound experimental methodology. The major theoretical debate has revolved around which sensory modality should be stimulated, and when and how much intervention is appropriate. In spite of recommendations that intervention should reflect the ontogeny of the nervous system, mimic either the intra- or extrauterine environments, or be driven by the impact that stimulation has on behavior, there appears to have been no concerted effort to develop a comprehensive approach toward the stimulation of premature infants.

This proposed presentation introduces a sequential multimodal approach (SMA) that takes into account the effects that the various forms of intervention (i.e., auditory stimulation through recorded heart beat, oral stimulation through a pacifier, vestibular stimulation via an oscillating waterbed, tactile stimulation through massage) have upon behavioral state (i.e., whether the form of stimulation elicits quiescence or arousal). The goals of SMA, which are reflected by the sequence of stimulation, include (1) promoting state regulation (via rapid vestibular stimulation, auditory and oral stimulation), (2) facilitating the infant's interface with the environment (via slow vestibular stimulation and oral stimulation), and (3) enhancing general neurobehavioral development (via tactile/kinesthetic stimulation).

The infant's point of entry into this treatment model would depend on its level of behavioral instability. Furthermore, the infant's reaction to each treatment stage would determine its progress along the algorithmic pathway: An adverse reaction to a newly introduced stage would result in a return to the previous form(s) of stimulation (or in more serious cases, no stimulation at all).

This proposed presentation also provides findings of a large-scale investigation that is currently in progress that is testing the SMA treatment components. This study is attempting to determine (1) if fast vestibular stimulation (FVS: waterbed oscillations of 25 times per minute) promotes more frequent and deeper sleep states; (2) if slow vestibular stimulation (SVS: waterbed

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oscillations of 16 times per minute) increases periods of quiet alertness; (3) if tactile/kinesthetic stimulation (T/K: daily massage and passive limb movement) promotes greater arousal and motoric output; (4) if the treatments have a differential effect upon growth parameters, behavioral organization, and neurobehavioral development; and (5) whether the gestational age and birth weight of the infant interact with the treatment effects. Preliminary results are presented from a small sample (final N will equal 90 Ss) of low birthweight and very low birthweight premature infants. Behavioral observations during treatment and nontreatment periods provide initial support or refutation of the hypothesized effects of the interventions upon behavioral state. Neurobehavioral maturation is examined pretreatment and posttreatment with an adaptation of the Brazelton. Posttreatment findings from treated preterms are compared against control preterms and full-term neonates. It is expected that these preliminary findings will support the SMA treatment model for premature infants.

Regional Fate in the Developing Cerebral Cortex Is Specified Prior to the Final Cell Cycle

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The differentiation of the cerebral cortex involves the formation of laminae in the radial domain and the parcellation of functional areas in the tangential domain. The fate of neurons destined for different cortical layers is regulated in the precursor population through a cell cycle-dependent mechanism, but it is unclear whether a similar process governs the determination of neurons forming different cortical areas.

We previously defined an interaction between collagen type IV and transforming growth factor (TGF)a that regulates the expression of the an area-specific phenotype, the limbic system associated membrane protein (LAMP), by cortical neurons in vitro. When grown in the presence of this inductive signaling system, even precursors derived from nonlimbic regions of the cerebral wall express LAMP. In contrast, when grown in a noninductive environment, only precursors derived from presumptive limbic regions express LAMP. In this study, we examine the mechanisms underlying the induction of LAMP in the nonlimbic precursors using retroviral labeling to identify discrete clones. When grown in a noninductive environment, an individual progenitor will give rise to neurons of the same molecular phenotype. Thus, daughter cells are able to retain the memory of an area-specific inductive signal received in vivo, even though they may have passed through as many as three cell cycles in culture. This memory, however, is not irreversible. Precursors that progress through at least two complete cell cycles while exposed to TGFa/collagen type IV express LAMP; progenitors that pass through only one cell cycle fail to respond to the inductive signals.

These experimental studies are consistent with the concept that a general mechanism of early fate determination, dependent upon environmental signals acting at the level of the precursor cells, regulates major aspects of early cortical regionalization. It is likely that these signals are spatially segregated within the ventricular zone and operate over the entire time of neurogenesis to produce neurons with a molecular feature unique to a discrete cortical domain.

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The Development of Sleep Regulation in the Rat

M.G. Frank, and H. Craig Heller

Adult mammalian sleep is homeostatically regulated. Total sleep deprivation in adult animals transiently increases rapid-eye-movement (REM) sleep time, and nonREM sleep electroencephalograph (EEG) slow-wave activity (SWA) during recovery sleep. Thus, the increase of REM sleep time and nonREM SWA following sleep deprivation provides indices of sleep homeostasis, which is presumably related to sleep function.

The development of sleep states and sleep regulation was investigated in neonatal rats using a sleep recording system that minimized the effects of maternal separation and permitted the continuous collection of EEG and electromyograph (EMG) sleep records. Our conclusions are that neonatal "active sleep" is not homologous with REM sleep and represents a common precursor sleep state for both REM and nonREM sleep. Moreover, nonREM SWA is the first EEG characteristic of adult sleep to appear during development. After characterizing sleep development, we used brief periods of sleep deprivation to investigate the development of sleep homeostasis. P12-P24 rats were sleep deprived for 3 hours using forced locomotion or gentle handling. Sleep deprivation in P12-P20 rats significantly increased nonREM sleep time during recovery, but did not increase nonREM SWA until P24. REM sleep did not significantly increase (rebound) during recovery until P16. These findings suggest that (1) nonREM sleep is regulated very early in development, (2) nonREM sleep regulation precedes REM sleep regulation, and (3) neonatal rats cannot intensify SWA in response to sleep deprivation and compensate by increasing total nonREM sleep time. The presence of sleep regulation in neonates, coupled with the large amounts of sleep in the neonatal period, suggests an important role for sleep in neonatal mammals.

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Quantitative Magnetic Resonance Imaging of Normal Human Brain Development: Ages 4–18

J.N. Giedd and J.L. Rapoport

Many of the most severe neuropsychiatric disorders of childhood onset may reflect deviations in normal brain development. To provide a contrast group from which to assess pathologic development, as well as to elucidate neuroanatomical characteristics of normal brain development, 120 healthy boys and girls, aged 4 to 18, were recruited from the community for participation in a quantitative magnetic resonance imaging study of the cerebrum. Images were acquired on a GE 1.5 Tesla Signa Scanner and analyzed with a variety of computer-assisted image analysis programs. Gender-specific maturational changes were noted in several regions. Specifically, there were age-related decreases in the size of the caudate nucleus (males only) and age-related increases in the sizes of the left amygdala (males only), corpus callosum (males and females, most pronounced in posterior regions), right hippocampus (females only), and lateral ventricles (males only). Male brains were approximately 9 percent larger than female brains across all ages from 4 to 18. This was significant even when controlling for height and weight. When adjusting for this difference in total cerebral volume, females had a relatively larger caudate than males, while males had a relatively larger putamen, globus pallidus, and lateral ventricle volume. Laterality effects were noted with significant right-greater-than-left differences for the amygdala, caudate, cerebral hemisphere, hippocampus, and temporal lobe. Left-greater-than-right differences were observed for the globus pallidus and lateral ventricles. Interactions between these gender-specific developmental patterns and environmental influences may be related to the observed gender differences in prevalence, age of onset, and symptom profiles in nearly all childhood neuropsychiatric disorders.

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Enduring Effects of Early Abuse on Locomotor Activity, Sleep, and Circadian Rhythms

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Stress stemming from early abuse has been hypothesized to be an etiological factor in the genesis of several psychiatric disorders. Recent studies suggest that childhood abuse can exert enduring neurobiological effects. The purpose of the present study was to ascertain the biobehavioral effects of early abuse on locomotor activity levels, sleep initiation and maintenance, and circadian rhythms. In particular, we sought to test the hypothesis that early abuse would be associated with delayed sleep initiation and reduced sleep efficiency. Further, we sought to determine whether abused children with posttraumatic stress disorder (PTSD) differed from abused children without PTSD in their activity and circadian measures.

Nineteen unmedicated prepubertal children with documented abuse were compared with 15 nonabused normal controls and 10 depressed children. All subjects received a complete semistructured diagnostic interview (K-SADS-E). Motionlogger actigraphs collected activity data for 72 continuous hours in 1 minute epochs. Nocturnal activity levels in abused children were twice as high as nonabused depressed or normal children. Use of sleep estimation algorithms revealed that these disturbances stemmed from protracted sleep onset latency and diminished sleep efficiency. Both physical and sexual abuse significantly disturbed sleep; however, physical abuse exerted a greater impact than sexual abuse. Neither PTSD nor depression exerted significant effects on sleep. Abused children had elevated activity levels, largely due to those children with PTSD, who were 12 percent more active than normal. Abused children with PTSD also had robust and preserved circadian rhythms, while abused subjects without PTSD had dysregulated rhythms. Development of PTSD was strongly correlated with age at onset of abuse, with earlier abuse associated with greater likelihood of PTSD. Overall, activity profiles of abused children with PTSD were similar to those observed in ADHD, and in this sample 38 percent met criteria for ADHD (vs. 0 percent of abused children without PTSD). In contrast, abused children without PTSD had dysregulated activity profiles similar to those previously observed in depressed children.

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Contextual, Behavioral, and Physiological Processes: The Study of Girls' Entry Into Adolescence

J.A. Graber and J. Brooks-Gunn

The purpose of the present paper is to outline conceptual models that integrate several areas of research in order to predict girls' adjustment during the transition into adolescence. Preliminary analyses testing these models is also included. The present study is based on data collected in the first year of a 4-year longitudinal project examining girls' development from 3rd to 6th grade. This investigation looks at how early pubertal changes amplify another physiological process, reactivity to stress. Investigations of reactivity to stress in infants and young children suggest that stable individual differences exist among children across those periods and are associated with unique patterns of physiological responses to stress. Middle childhood presents new challenges, especially at school and in the peer group. At this time, it is unknown how children respond behaviorally and physiologically to such stresses. Focusing on girls during this time is important as girls are more likely than boys to begin pubertal development during middle childhood and, in particular, girls who mature earlier than their peers are much more likely to experience such changes with less anticipation or preparation for them. Evidence suggests that African-American girls mature earlier than white (non-Hispanic) girls; yet there is virtually no information on the influence of early pubertal timing on adjustment in nonwhite girls.

Girls and their families are from integrated, working, middle-class communities. Of the children presently enrolled in the study, 35 percent are white (non-Hispanic), 35 percent are African American, and 30 percent are Hispanic American (predominantly white Puerto Rican). Over half of the children (58 percent) live in the same home with both biological parents. Median family income is $37,500, and 35 percent of mothers do not work outside of the home. Thirty-six percent of fathers and 20 percent of mothers had less than a high school education. Data were collected via child self-report, mother report on self and daughter, father self-report, and a videotaped home visit that included a series of potentially challenging tasks, a child interview, and mother-child interactions. In addition, girls collected saliva and urine samples at home over a 3-day period. For the purposes of the present paper, measures of pubertal development, reactivity to stress, and temperament and adjustment are used. Analyses address the following questions: Do various indices of pubertal

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processes render girls at risk for adjustment problems? Do patterns of reactivity as exhibited in the cold challenge amplify the potential effects of early pubertal development? Are the associations among puberty, reactivity, and adjustment similar or different for white (non-Hispanic), Hispanic, and African-American girls?

Developmental Analysis of Immune Reactivity, Social Stress Reactivity, and Social Behavior in Selectively Bred Mice

D.A. Granger, K.E. Hood, S.C. Ikeda, and C.L. Reed

Exaggerated cellular immune activation, or central nervous system (CNS) sensitivity to inflammatory products, or both, are known to influence biobehavioral development. Studies have yet to examine individual differences in susceptibility to the behavioral or CNS effects of peripheral immune activation. We have examined whether individual differences produced by selective breeding in mice influence the effects of the acute phase response induced by lipopolysaccharide (LPS) on central monoamines, hypothalamic-pituitary-adrenal (HPA) axis products, thermoregulation, and social behavior. Subjects in all studies were ICR mice selectively bred for isolate-housing induced intermale aggression. In the first study, low-aggressive (low-line) and high-aggressive (high-line) mice received i.p. either saline or 2.5 mg/kg of LPS (Sigma; E. Coli, O111.B4). In contrast to the low-line mice, high-line mice exhibited a rapid, large (15 °C), and persistent LPS-induced temperature decline. In a second study, low- and high-line mice were administered i.p. saline, 0.25 mg/kg, 1.25 mg/kg, or 2.5 mg/kg of LPS. Results revealed a dose-dependent LPS-induced increase in spleen weights and corticosterone levels for the high- but not the low-line mice. In a third study, low- and high-line mice received i.p. either saline, 0.25 mg/kg, or 1.25 mg/kg of LPS. Core temperatures and body weights were monitored before and 1 and 2 h postinjection. Social behaviors were coded from videotaped social challenge tasks. Brains, spleens, and sera were collected for later assay. Results revealed that LPS reduced aggressive behavior (attacks) in the high-line and increased freezing (prolonged immobility) in the low-line mice. Endocrine and monoamine assays are pending. Taken together, these findings support strong individual differences in susceptibility to immune challenge. Current research presents a developmental analysis. In the fourth study, neonates received 0.05 ug/kg LPS i.p. on postnatal days 5 or 6. Results to date reveal a short-term developmental effect of LPS in high-line mice. LPS-treated high-line animals showed slower growth (indexed by weight gain) at 24 and 72 h postinjection. These subjects are to be assessed at 45 days of age for differences in social behavior and HPA axis reactivity to social and immunological stimuli. These results are the focus of the proposed presentation.

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A Longitudinal Study of Children of Depressed Mothers: Psychobiological Findings Related to Stress

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Children of mothers experiencing depression are at risk for a variety of behavioral and emotional difficulties, including problems in self-control, poor peer relationships, academic difficulties, attention problems, and affective disorder. Emotional disturbances associated with maternal depression can be seen even in young infants, who tend to be withdrawn and less active and to have diminished positive affect. We found that, compared to infants of nondepressed mothers, 14-month-old infants of depressed mothers showed reduced left frontal brain activation during play with their mothers and during an alert baseline condition. A number of neuropsychological studies have linked the frontal lobes to emotional expression and regulation, and reduced left frontal brain activity is found in depressed adults. Furthermore, these infants showed increased heart rates while interacting with their mothers and a familiar experimenter.

We have been following this sample of children and their mothers to examine a number of psychophysiological indices of emotional development in relation to changes in mothers' depression status over time. These indices include child EEG activity, autonomic activity, and sleep patterns. In addition, we were interested in whether these children would show elevations in another measure linked to stress and depression, salivary cortisol. Recently, we found that children of mothers who were depressed at both 14 and 39 months exhibited elevated salivary cortisol as compared to those children whose mothers were consistently nondepressed or depressed only at 14 months. This pattern parallels our previous finding that EEG patterns remain stable in children of chronically depressed mothers but return to more typical patterns when maternal depression remits. The long-range goal of this study is to determine the extent to which psychobiological measures are predictive of children's emotional disturbances and behavioral problems in early childhood.

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Prenatal Stress Increases Adult Aggressive Behavior and Adrenocortical Reactivity in Mice

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To explore the possibility that the frequency or developmental timing of prenatal stressful events is linked to individual differences in subsequent biobehavioral responsiveness to social challenge, we studied the developmental effects of prenatal stress in ICR mice. We asked three questions. What are the effects of prenatal stress on the development of social behavior in offspring? Is there a sensitive period during fetal development for prenatal stress effects? Are aggressive behavior and adrenocortical reactivity after prenatal stress exposure linked?

The study compared offspring from groups of outbred mice that had higher or lower levels of prenatal stress in the breeding cage. Prenatal stress here consists of aggressive behavior among members of 15 breeding groups, each of which included 2 to 4 novel unrelated females and 1 to 2 novel unrelated males. Event sampling during daily 40-minute observation periods over 16 days provided measures of prenatal stress (number of attacks observed in the breeding cage). Subjects were 27 male mice from 14 pregnancies. Low levels of prenatal stress (0 or 1 attack during the prenatal period) characterized 6 pregnancies. High prenatal stress during early pregnancy (12 to 23 total attacks in prenatal days 1 to 4) characterized 5 pregnancies. High prenatal stress during late pregnancy (43 to 55 total attacks during prenatal days 12 to 15) characterized 3 pregnancies. Twenty-one days after birth, the subjects were weaned and isolate housed. At 45 days of age, social behavior and adrenocortical activity were assessed. Subjects were videotaped with an unfamiliar male partner for 10 min in a dyadic interaction test. Four categories of social behavior were coded: Attacks (vigorous biting and wrestling), freezes (prolonged immobility), withdrawal responses (kick or startle), and sniffs (exploratory social behavior).

Analyses revealed that high levels of prenatal stress are correlated with high number of attacks during the dyadic test. No relationship was found for other behaviors. Late prenatal stress predicts high levels of attacks in adulthood. Early prenatal stress shows no relationship with attack. There are no correlations between behaviors and corticosterone levels. However, regression analyses show an interaction: High prenatal stress subjects which at the time of testing showed high levels of attacks had the highest corticosterone response to social challenge, indicating their high level of stress at the time of testing.

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These findings show strong support for the developmental plasticity of aggressive behavior. Prenatal stress, especially in late pregnancy, may have implications for human development. In particular, risk factors for the development of antisocial behavior in children may include the fetus' experience of domestic violence before birth.

Neonatal Hypothalamic-Pituitary-Adrenocortical System's Response to Repeated Stressful Stimulation

M. Israelian and E. Emory

Salivary cortisol values were assayed in newborns in order to study the hypothalamic-pituitary-adrenocortical (HPA) system's response to repeated, stressful stimuli. Two distinct stimuli were employed: (1) pricking of the neonate's heel (heelstick) and (2) handling of the infant via a neurological exam, the Neurological and Adaptive Capacity Score (NACS). Subjects were assigned to two groups, a heelstick only group, which underwent an admission and discharge day heelstick, or a heelstick-handling group, which underwent an admission day heelstick and a discharge day NACS. Each group consisted of 10 subjects (5 male, 5 female). Saliva was collected from the neonates immediately before and 20 minutes after each experimental manipulation in order to obtain baseline and poststimulation cortisol values. Paired sample t-tests indicated that for the heelstick-handling group, the mean baseline cortisol value for Day 1 (1.76 μg/dL) was significantly higher than that for Day 2 (0.66 μg/dL), $t(df=9)=7.39$, $p<0.01$. The elevated baseline cortisol values in this group may highlight what is known as the "birthday effect"—elevated levels of cortisol at birth tend to decrease following a 24-hour postpartum period. An independent groups t-test conducted on the mean prestimulation cortisol values for both groups on Day 2 indicated that the heelstick prestimulation cortisol level (1.45 μg/dL) was significantly higher than the handling prestimulation cortisol level (0.66 μg/dL), $t(df=18)=-2.51$, $p<0.05$. It is hypothesized that this difference may reflect contextual or temporal learning effects in newborns. It is possible that when the infants were stuck on the admission day, the nurse and the activities that precede the heelstick procedure became paired with the painful effects and subsequent rise in cortisol value to the heelstick. Thus, subsequent exposure to the nurse and the heelstick context possibly came to elicit the elevated cortisol value, thereby explaining the elevated prestimulation cortisol level for the discharge heelstick. As such, our data may possibly reflect one-trial conditioned aversion in newborn infants. The data further suggest that regardless of the day of manipulation and the initial level of the prestimulation cortisol, the final poststimulation cortisol responses to the heelstick do not differ from one another, $F(2, 28)=0.53$, $p>0.05$. The upper limits of the HPA mediated cortisol response may be observed at birth. A one-way ANOVA revealed that the prestimulation cortisol values for both groups differed by day of testing

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F(3,37)=7.68, p<0.01. A series of planned comparisons performed on the data found no group differences for the heelstick manipulation on Day 1. However, Day 2 baseline values were significantly lower for the handling manipulation in the heelstick-handling group than for the heelstick manipulation for all other baseline values. Similarly, a second one-way ANOVA revealed that the mean poststimulation cortisol values differed by day of testing F(3,36)=3.5, p<0.05. Once again, there were no group differences on Day 1 for the heelstick manipulation. Also, Day 2 mean poststimulation cortisol values for the heelstick-handling group (0.82 µg/dL) were significantly lower than those for the heelstick only group (1.43 µg/dL), p<0.01. The data suggest that the heelstick manipulation is a more salient and stressful stimulus than the handling manipulation, in that poststimulation cortisol values for heelsticks were significantly higher than those for handling. Finally, the data suggest that the two groups were virtually identical with respect to their baseline cortisol levels prior to the first heelstick manipulation on Day 1 of testing. It is possible that individual differences account for the observed divergence of poststimulation values on Day 1 and Day 2. In addition to these individual differences, maturation and contextual effects may further account for the observed divergence on Day 2.

Singing Is Accompanied by Increased Gene Expression in Selected Nuclei of the Avian Song System

E.D. Jarvis and F. Nottebohm

Birdsong advertises an individual's identity, breeding predisposition, and territorial ownership. Whereas all songbird species studied so far learn their song by reference to external models, they differ in the timing of this learning. For example, male canaries continue to modify their song year after year, and most of this change occurs in late summer and early fall, after the end of the breeding season. By contrast, zebra finch males acquire a new song only once, before sexual maturity. However, for both these birds the acquisition and production of learned song occurs under the control of the same set of nuclei. Interestingly, whereas some of the nuclei of the song system (e.g., Area X, DLM and lMAN) are involved just with song learning and are not necessary for the maintenance of learned song, other nuclei (e.g., HVC and RA) are involved in both the acquisition and production of learned song.

In this study, we explored the relation between singing and expression of an immediate early gene transcription factor, ZENK, that has been implicated in the process of learning. Male canaries and zebra finches were stimulated to sing by exposing them to tape recorded playbacks of conspecific song and/or by presenting them with females. After a period of 30 min the birds were killed, their brains were removed, and ZENK expression was analyzed by in-situ hybridization. We found that birds who sang within the 30 min period had increased levels of ZENK mRNA in specific nuclei of the song system. Both canaries and zebra finches showed increased expression in HVC and RA; however, only canaries showed increased expression in Area X and lMAN. In both species, the amount of ZENK expression was correlated with the amount of singing. Birds that did not sing at all had no detectable expression of ZENK in the song nuclei. Moreover, cutting the nerve to the syrinx did not prevent an increase in ZENK expression in those birds that attempted to sing, though they were unable to produce the normal sounds of song. We believe that our observations raise basic questions about how production of a learned skill affects the expression of genes that may be involved in the consolidation of this skill.

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Infant Abuse Runs in Families of Group-Living Pigtail Macaques

D. Maestripieri and K. Wallen

Nonhuman primates are the only animal model for studying the etiology of child abuse and neglect. Research on infant abuse in primates, however, has made virtually no progress since the work of Harlow and coworkers in the 1960s. Here we report the first genealogical data on the spontaneous occurrence of infant abuse and neglect in a large population of group-living primates. The data were obtained from the Animal Record Files of the Yerkes Regional Primate Research Center. Maternal abuse and neglect of offspring was studied in 6 families of pigtail macaques (*Macaca nemestrina leonina*), numbering a total of 399 individuals, over a period of 33 years (1962-95). The monkeys have lived in large social groups at the Field Station of the Yerkes Center. Eleven cases of neglect and 37 cases of abuse were reported. Neglect was a phenomenon largely limited to first-time mothers dealing with their young infants. In contrast, infant birth order and age were not risk factors for abuse. Abuse was most likely to occur in some families and in closely related individuals. Infants whose siblings had previously been abused were themselves especially at risk of abuse. Maternal health was not a risk factor for abuse, and only one abusive mother was herself abused as an infant. The sex of abused infants and the physical patterns of abuse varied between families suggesting that abuse is not a unitary phenomenon. These and other characteristics of the spontaneous occurrence of infant abuse in socially living macaques suggest that this phenomenon could represent a good animal model.for studying the etiology of child abuse and neglect.

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Socioemotional Disturbances After Early Versus Late Medial Temporal Lobe Lesions in Rhesus Monkeys

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Monkeys with neonatal damage to the medial temporal lobe (amygdala, hippocampus, and underlying cortex) displayed, at the age of 6 months, socioemotional disturbances characterized by lack of social interactions, lack of dominance, and increased amount of both locomotor stereotypies and self-directed activities, when they were paired with age-matched normal controls. The socioemotional behavior of these dyads was reassessed when they reached the age of 4–5 years and were placed in a large enclosure. These dyads showed a severe lack of social interactions as compared to dyads consisting of adult normal controls, raised in a similar way. In addition, adult monkeys with neonatal lesions displayed increased amounts of both self-directed activities and manipulation and lack of dominance during competition for food rewards. These findings indicated that early damage to the medial temporal lobe structures results in long-lasting alteration of socioemotional behavior. To determine whether this degree of behavioral abnormality would follow the same damage to the mature brain, we assessed the behavior of monkeys given the same bilateral medial temporal lobe removals in adulthood and compared it with the behavior of both normal adult monkeys and the adult monkeys with neonatal damage. Each operated adult monkey was placed in a large enclosure with its peer-reared normal control, and their behavior was videorecorded for two 5-minute intervals during 5 consecutive days, both at 2 and 6 months after surgery. Similarly, each adult normal monkey was paired with another normal control and videorecorded twice. Unlike the monkeys with neonatal lesions, the monkeys with adult lesions displayed normal amounts of self-directed activities and manipulation. The amount of social interactions in the dyads including the monkey operated in adulthood was slightly but significantly lower than that in the age-matched normal dyads, but still significantly greater than that in the dyads including the adult monkey operated in infancy. These results indicate more profound changes in socioemotional behavior when the medial temporal lobe is damaged in early infancy than when it is damaged in adulthood.

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Deafness Drives Development of Attention to Change in Visual Field

T.V. Mitchell

Deaf (n=37) and hearing (n=37) subjects aged 6-7, 9-10, and 18+ participated in a visual search experiment designed to test the hypothesis that vision in the deaf becomes specialized over developmental time to detect change in the visual field. All children, regardless of hearing status, should attend to change in the visual field. However, the differing developmental experiences and sensory "tools" between the two groups create different demands on their visual systems. Hearing individuals are capable of monitoring the world auditorially while attending to task-relevant information visually and therefore develop the ability to ignore many changes in the visual field. Deaf individuals must both monitor the world and attend to task-relevant information visually and therefore must maintain attention to change in the visual field. Subjects in this experiment completed two visual search tasks in which they searched for a uniquely shaped target in the presence of two irrelevant stimulus manipulations (color or motion). This manipulation was applied to the target on half the task trials and to a distractor on the other half. Attention to the irrelevant manipulations will create differential reaction times (RTs) when the target is manipulated versus when a distractor is manipulated. Results indicated divergent development between the two groups. Both deaf and hearing children produced differential RTs in the two tasks, while only deaf adults attended to the task-irrelevant changes. Further, hearing subjects were more affected by apparent motion than by color, while deaf subjects were equally affected by both manipulations. Results are discussed as compensatory changes in visual processing following auditory deprivation.

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Differential Effects of Life Orientation on Cortisol in Pregnant and Nonpregnant Adolescents

E. Murowchick and B.K. Worrall

The purpose of this report is to examine salivary cortisol excretion patterns within a pregnant adolescent and a nonpregnant comparison group. Plasticity is important during adolescence for two reasons. First, adolescence is a stage relatively early in the development of humans when physiological systems are evolving. Second, adolescence brings an explosion of development in biological, cognitive, and social domains. In the current sample, the recency of puberty and the concurrent onset of pregnancy in half the sample provide the opportunity to assess plasticity in reactivity of the hypothalamic-pituitary-adrenal axis (HPA) in relation to their life orientation (optimism vs. pessimism). The hypothesis tested was that there would be a differential effect of cortisol on the environment for the two groups.

The data were from a longitudinal study of the biobehavioral development of adolescents. The sample consisted of 77 pregnant and 57 nonpregnant rural girls aged 13-19, matched by age and socioeconomic status. Data from the first time of measurement (11-16 weeks of gestation) were used in the present analyses. The physiological data consisted of the initial sample of cortisol (0 minutes) and the percent change in cortisol across five samples obtained every 20 minutes. The behavioral data consisted of the Life Orientation Test (LOT). Results indicated a differential effect of the initial value cortisol variable on the LOT for the two groups. In the comparison nonpregnant sample, there was a significant effect of the initial value of cortisol on the LOT, $F(1,53)=4.23$, $p=0.04$, but there was not a significant effect in the pregnant group, $F(1,76)=0.28$, $p=0.65$. For the LOT predicted by the percent change in cortisol variable, neither group obtained statistically significant results. Thus, it seems that the transition of adolescence combined with pregnancy, at least during the first trimester, buffers the impact of initial value cortisol on the environment, whereas adolescence alone does not buffer the individual. In conclusion, there is less plasticity in the HPA axis in the pregnant adolescents. Evolutionary forces may have supported a system that prevents outside stress from playing as large a role on their day to day existence.

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Differential Genetic and Environmental Contributions to Stability and Change: A Comparison of Three Social Behaviors

J. M. Neiderhiser

During adolescence there are numerous changes internal to the adolescent such as the onset of puberty and changes in cognitive functioning, and external to the adolescent in terms of the social reactions of others to the adolescent and changes in others' expectations. These changes may also be explained in terms of genetic and environmental contributions to change and stability. The present study consists of the complete longitudinal sample from both waves of the Nonshared Environment in Adolescent Development (NEAD) project (395 families). The NEAD project includes adolescent siblings residing in never divorced families (MZ and DZ twins and full siblings) and in step-families (full, half, and unrelated siblings). The sample was assessed on two measurement occasions, 3 years apart. Composite measures of parent reports, adolescent self-reports, and observer ratings of three adolescent social behaviors (antisocial behavior, autonomy, and global self-worth) were examined. Each measure showed a different pattern of genetic and environmental contributions to stability and change, suggesting differential pathways for each domain of adolescent social behavior. For example, genetic influences are important for both change and stability in antisocial behavior. Stability and change in global self-worth, on the other hand, are influenced by primarily nonshared environmental factors, although there are also some genetic contributions to change. Finally, genetic and shared environmental influence contribute nearly equally to stability and change in autonomous functioning.

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A New Model of Olfactory Imprinting in Salmon

G. Nevitt

Salmon are a highly tractable model species for examining olfactory learning and memory. These fish have an acute sense of smell and also imprint and home to experimentally controllable olfactory cues that are learned during a well-defined period of development, the parr-smolt transformation (smolting). However, the neural basis of olfactory imprinting in salmon has remained a neuroethological puzzle for over 40 years.

Proponents of olfactory imprinting have assumed that the olfactory memory for the home stream resides entirely in the central nervous system. I have challenged this assumption and propose instead that some component of the olfactory memory is retained in the olfactory receptor cells themselves. I have further suggested that the physiological and structural acquisition of this memory is linked to smolting, because it is regulated by the elevated thyroxine (T_4) levels occurring at this critical period. The promise for this new theory comes in part from recent patch clamp data of olfactory receptor cells isolated from imprinted fish. These results show an increased sensitivity of peripheral olfactory receptor cells to the imprinted odorant.

I have developed a new model to serve as a framework to examine how the imprinted olfactory memory gets established. This model suggests that: (1) During the parr-smolt transformation, thyroid hormones (T_3 and T_4) promote a proliferation of olfactory receptor neurons that are sensitive to a wide variety of odors. (2) Receptor cells that are most active (i.e., responsive to the odorants present in the environment) survive, while others die. Selective survival may involve competition for synaptic targets. (3) This punctuated proliferation and selective survival of olfactory receptor cells triggers a reorganization of glomerular structures within the bulb.

The idea that olfactory receptor cells may play some role in the imprinting process is a thoroughly novel concept, begging further investigation with modern technology. If it can be shown that T_4 is influential in establishing this memory, such a result will certainly precipitate extensive work in this area, from the behavioral to the molecular level.

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Psychological Differences Between Maltreated and Nonmaltreated Children's Processing of Emotional Stimuli: The Effects of Early Experience

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Theories of emotional development postulate that aberrant early experiences may alter the processing and organization of emotions. This perspective includes emotion systems among those domains that develop based upon information that the nervous system receives from the environment. For example, the literature on child maltreatment indicates that prolonged stress and threat impacts broadly upon the behavior of children. Because maltreated children develop within environments marked by threat and stress, negative affective cues are likely to represent highly meaningful stimuli for them. We used the event-related potential (ERP) to explore the cognitive processing of happy, angry, and neutral faces in maltreated and nonmaltreated children. The ERP is an index of central nervous system (CNS) functioning thought to reflect underlying neurological processing of discrete stimuli and is particularly useful in linking brain physiology to psychological processes such as attention, memory, and categorization.

ERPs were recorded from 23 maltreated and 21 nonmaltreated children ranging from 7 to 11 years of age. The stimuli used were slides of Ekman photographs depicting a single model posing a happy, angry, or neutral facial expression. In each of two conditions, children were asked to press a button in response to either a happy or angry face, which was designated as a target, while ignoring the other two faces. Nonmaltreated children exhibited comparable amplitude of the P3b ERP component to target and nontarget faces in both the angry target and the happy target conditions. In contrast, maltreated children displayed increased P3b amplitude to all stimuli presented in the angry as opposed to the happy target condition.

This increased CNS responsiveness and deployment of cognitive resources when attending to negative affect may reflect the activation of mental representations. Such activation may trigger adaptive responses to meet the challenges presented by these children's threatening environments, but may also place these children at heightened risk for the development of psychopathology. These data also raise compelling questions about the role of experience in the development of emotion regulatory structures. Emotion systems have been postulated to function as associative networks wherein input that matches significant mental representations activates memory systems. P3b amplitude,

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in this context, may mark the match of facial stimuli with more complex emotional memories. The present psychophysiological results provide support for such experience-dependent developmental processes.

Birthweight and Gestational Age Outcomes From Antepartum Nonstress Test

F. Powell and E. K. Emory

Despite the introduction of fetal monitoring more than 30 years ago, surprisingly few developmental studies have been reported. In recent years, fetal monitoring of heart rate and movement have defined the standard by which prenatal diagnosis is made. Now a regular part of antepartum (before labor) and intrapartum (during labor) testing, fetal reactions provide predictive and clinical management data from which significant clinical decisions are made.

One particularly useful aspect of fetal surveillance (through heart rate and movement recording) is the Nonstress Test (NST), which, if reactive, is usually indicative of an intact nervous system. Nonreactive tests (e.g., those that fail to meet fetal heart rate response criteria) are more often associated with fetal compromise, especially during late pregnancy. The purpose of this study was to examine the association between reactivity to NST and fetal neurologic/physiologic outcome.

One hundred five fetal subjects who were administered at least one NST were evaluated on the basis of gestational age, birthweight, ponderal index, body length, and 1-minute and 5-minute APGAR scores. Seventy-five subjects had a reactive test and 30 subjects had a nonreactive test. Utilizing logistic regression to control for maternal age and gestational age at time of NST (only the first test was analyzed), fetal subjects with a nonreactive test had lower birthweights, $p<0.001$, shorter gestations, $p<0.005$, and smaller weight-to-length ratios (ponderal index) $p<0.05$.

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Genetic Constraints on the Developmental Plasticity of Behavioral and Neuroendocrine Systems and Their Coupling in a Stressful Situation

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A selective breeding program produced lines of male mice that differ markedly in their propensity to attack when exposed to an unfamiliar male following isolation rearing. While high-aggressive animals attack rapidly and fiercely in a dyadic test, low-aggressive animals freeze and become immobile instead. In the first case, stimulus intensity is controlled by acting upon the stimulative source or by escaping it. In the second case, this control is achieved internally by modulating the neuroendocrine systems that regulate the emotional impact of perceived stimuli. It has been reported that the latter strategy provides for greater flexibility in behavior and more rapid adjustments to environmental changes.

The present experiment was guided by the hypothesis that a coupling between behavioral and neuroendocrinological responses to novel stimulation would be more characteristic of the low-aggressive line. It was further hypothesized that these genetically based differences would also impose differential constraints on the sensitivity of the developing systems to variations in stimulative conditions.

Male mice from each line were either handled postnatally or left undisturbed until 21 days of age, at which point they were weaned and assigned to either group or isolation rearing until they reached the age of testing. At Day 56, all subjects were singly placed in an open field arena and observed for 10 minutes. Thigmotaxis, unsupported, and supported upright rearing were recorded. Circulating levels of corticosterone (CORT) and testosterone (TEST) were determined for each subject 20 minutes after the test. As expected, similar patterns of activation in the HPA axis were observed across experimental conditions in the high-aggressive line. By contrast, the experience of handling significantly reduced CORT responses in the low-aggressive line, and those levels were lowest among subjects that had been subsequently reared in groups. The same manipulations affected patterns of activation in the HPG axis in the two lines, albeit to a greater extent in the low-aggressive line. Also, as hypothesized, correlations between neuroendocrine activation and behaviors reached significance in the low-aggressive line only. Specifically, supported rears were positively related to TEST measures, and thigmotaxis was negatively related to CORT levels. No such relationships were evident in the high-aggressive line.

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This result raises questions concerning the generality of thigmotaxis as a measure of emotional reactivity. Indeed, the coupling observed in the low-aggressive line between behavioral activity and neuroendocrine activation suggests that both wall-hugging and rearing upright against the side of the arena may simply constitute exploratory behavior. Finally, a negative relation between CORT and TEST was observed in the high-aggressive line only. The greater independence of the two hormonal systems in the low-aggressive line is consistent with their respective coupling to different aspects of exploratory behavior. The fact that elevated levels of TEST alone appear sufficient to support exploratory activity, even when emotional stress is relatively high, provides a degree of flexibility in behavioral adjustment not available to the high aggressive line. Overall, our results confirmed the existence of genetic constraints on the sensitivity of the developing systems to variations in stimulative conditions and their coupling under challenging conditions.

The Development of Maladaptive Social Behavior in Children: The Contributions of Biology, Behavior, and Temperament

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There is a large corpus of evidence suggesting that children who remain shy and timid in response to novel social situations during the early school years fail to develop social competence and are at risk for depression and internalizing problems. We examined differences in social behaviors, salivary cortisol, startle eyeblink responses, and maternal ratings of temperament at age 7 in an unselected group of children, some of whom were classified by maternal report as shy at age 4. Behavior, salivary cortisol, and startle responses were indexed at age 7 during a laboratory task designed to elicit social evaluation. We found a significant relation between shyness at age 4 and adrenocortical and startle reactivity at age 7. Four-year-olds who were classified as shy exhibited augmented baseline startle responses and displayed a greater increase in salivary cortisol in response to the laboratory task at age 7 compared with their nonshy counterparts. This same subset of shy children also presented with a higher proportion of socially reticent behavior during peer play at age 4 than other children and continued to be reported by their mothers as shy during the early school years. We speculate that high levels of cortisol in shy children may induce changes in the central nucleus of the amygdala, exacerbating their fearfulness. The interrelation between cortisol and the amygdala in the development of maladaptive social behavior is discussed.

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Timing of Prenatal Stress Affects Neurobehavioral and Endocrine Responses in Rhesus Monkey Offspring

M.L. Schneider, E.C. Roughton, C.F. Moore, and A.S. Clarke

The increased prevalence of violence against women in American families has resulted in a growing public concern about the effects of prenatal stress exposure on child outcome. However, studies on the effects of prenatal factors are difficult, if not impossible, to conduct in humans because of the confounding of prenatal care, postnatal rearing environments, and substance abuse in human studies. We have developed a nonhuman primate model of prenatal stress exposure that enables us to manipulate the timing, duration, and intensity of the exposure. We have previously reported that psychological stress administered during mid-late gestation induced neuromotor deficits and attentional disorders, diminished cognitive abilities, enhanced stress reactivity, and developmental alterations in HPA-axis modulation in offspring.

We conducted a prospective longitudinal study of 29 rhesus monkey offspring whose mothers were exposed to one of three conditions: (1) 10 min psychological stress during early gestation (45-90 days postconception); (2) 10-min psychological stress during mid-late gestation (90-145 days postconception); or (3) undisturbed conditions throughout pregnancy. It is important to determine whether a sensitive period exists for exposure to prenatal stress, because teratogens often differ in their effects on offspring depending upon the timing of exposure.

Our results indicate that early-gestation stress constitutes a more significant danger to the fetus than does mid-late gestation stress. When infants were assessed repeatedly using a test battery modeled after the Brazelton Neonatal Behavioral Assessment Scale, we found that while infants from both the early- and mid-late-gestation stress conditions showed impaired attention and neuromotor maturity compared to controls, the early-stress group was significantly more impaired than the mid-late-stress group. Moreover, repeated observations of mother-infant behavior across the first 6 months of life indicated that while control dyads steadily decreased time spent in mutual ventral contact and nipple contact, early- and mid-late-gestation stressed mothers maintained high levels of these behaviors across time. Finally, infants from the early-stress group were observed to spend more time in sleep compared to other infants, and analysis of state behaviors indicated that they showed high levels of drowsy state. At 6

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months of life, when the infants were separated from their mothers, the early-stress group showed higher than normal ACTH responses 2 h after maternal separation. Hence, our data to date indicate that early gestation stress is more harmful to the fetus than stress induced later in pregnancy.

Maternal Hormones and Behavioral Development in Birds

H. Schwabl

The differential secretion of sex steroid hormones during critical periods plays a key role in the development of differences between the sexes in brain function and behavior. However, this cannot explain the variability in the behavior of individuals of the same sex, which can be as great as the behavioral differences observed between the sexes. Such within-sex differences in behavior may also be caused by differences in the exposure to steroid hormones during development, and one source of these hormones may be the mother. The yolk of canary *(Serinus canaria)* eggs contains variable doses of maternal testosterone that increase in each subsequently laid egg of a clutch. This maternal testosterone influences at least two aspects of development. (1) Hatchlings from eggs with high doses of maternal testosterone beg more vigorously for food and as a consequence grow faster. This may be caused either by a general metabolic effect of testosterone or by the enhanced development of sensory and/or motor control systems that underlie the food-begging behavior of the newly hatched chick. (2) Offspring from eggs with higher doses of maternal testosterone are more aggressive as adults regardless of their genetic sex. This suggests that maternal testosterone may have organizing developmental effects on brain and behavior analogous to those of sexual differentiation. Egg-laying animals thus provide an excellent model to study the contribution and interaction of genetic, maternal, and environmental factors in the development of behavioral differences among individuals.

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Estrogen Stimulates the Rapid Phosphorylation of Extracellular-Signal Regulated Kinase (ERK) in Organotypic Cerebral Cortical Explants

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Several factors are known to play significant roles in the development of the central nervous system (CNS). Of these, estrogen and the neurotrophins have been ascribed an important role in the developmental plasticity which ultimately leads to the molding of a mature CNS. Furthermore, the trophic importance of estrogen and the neurotrophins is not restricted to developmental processes but is also important for maintenance and perhaps cytoprotective effects during adulthood and in disease states. In view of the comparable effects of neurotrophins and estrogen on a variety of endpoints (such as neurite outgrowth and cytoprotection), along with the established observation of estrogen and neurotrophin receptor colocalization, we suggested that the two ligands, neurotrophins and estrogen may share a common signal transduction pathway. One prominent pathway employed by the neurotrophins is the MAP kinase pathway in which receptor activation by the neurotrophin leads to the rapid and prolonged activation of dual specificity (serine/threonine and tyrosine) kinases, the ERKs (or MAP kinase). As such, the aim of our study was to see if 17-βEstradiol could similarly activate the ERKs. We used the organotypic explant culture system to evaluate our hypothesis. Cerebral cortical cultures were explanted on P2 (P1=day of birth) and maintained in culture for 6 days supplemented with 10 nM estradiol (E2) and 100 ng/ml nerve growth factor (rhNGF) to simulate, at least partially, the neurotrophin and hormonal milieu the brain region would be exposed to in vivo. Following a washout period of 24 hours to establish a baseline for ERK activation, the cultures were pulsed with either E2 for 15, 30, 60, and 240 min or with a neurotrophin (100 ng/ml of NGF, brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3)) for 10 min. NGF-treated (5 min) and untreated PC12 cells served as positive and negative controls, respectively. Following Western analysis using an antiphosphotyrosine antibody, we observed that E2 resulted in the tyrosine phosphorylation of both ERK 1 and 2, although ERK 2 stimulation was more prominent. The stimulation of the ERKs was evident as early as 15 min and persisted up to 4 hours of treatment. NGF, BDNF, and NT-3 similarly resulted in a stimulation of ERK phosphorylation, with BDNF producing the strongest signal. To our knowledge, this is the first demonstration of an E2-induced stimulation of ERK in the CNS. Our results suggest that this rapid effect of E2

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may be an important aspect of potentially alternate or nonclassical (nongenomic) effects of estrogen that could mediate neuronal outgrowth and survival associated with developmental plasticity of the CNS.

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Effects of Maternal Deprivation and Stress on the Expression of Neurotrophic Factors, Neuropeptides, and Immediate-Early Genes

M.A. Smith and S. Levine

Prolonged separation from the mother interferes with normal growth and development and is a significant risk factor for adult psychopathology. In rats, maternal deprivation can disinhibit the hypothalamic-pituitary-adrenal (HPA) axis which is normally quiescent during the stress-hyporesponsive period from day 4 through 14. In an effort to understand how maternal deprivation may alter HPA axis sensitivity, we used in situ hybridization to measure changes in the expression of stress-responsive genes in male and female rats (postnatal day 12 and 20) after 24 h of separation from their mother. The mild stress of a saline injection increased the levels of the immediate-early genes (IEGs), c-fos and NGF1-β, in the cingulate cortex and hypothalamic paraventricular nucleus (PVN). Maternal deprivation potentiated the induction of these IEGs by stress at P12 but not at P20. Surprisingly, the basal level of corticotropin-releasing factor mRNA was decreased in the PVN by maternal deprivation at both P12 and P20. Preliminary evidence suggests that brain derived neurotrophic factor, which is important for the development and survival of neurons, was reduced by maternal deprivation in the hippocampus at P12. In contrast, nerve growth factor mRNA levels were increased by maternal deprivation. Alterations in transcription factors and neurotrophic factors during critical developmental periods likely influences processes such as apoptosis and neurite branching and thereby alters neural connectivity. Maternal deprivation during the stress hyporesponsive period in neonates may have long-term consequences for regulation of the HPA axis and may lead to abnormal behavior by permanently increasing stress sensitivity.

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Ontogeny of Human Forebrain Dopamine Receptors: Differential Expression of $[^3H]$–SCH23390 and $[^3H]$–YM09151–2 Specific Binding

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Dopamine receptor expression in human fetal forebrain (between 6 and 20 weeks of gestation) was measured using tissue-slice receptor autoradiography with the D_1-like and D_2-like antagonists $[^3H]$-SCH23390 and $[^3H]$-YM09151-2, respectively. Tissue sections were assayed in saturation studies and examined for age- and sex-related changes in B_{max}. We made the following observations: (1) the ages at which D_1- and D_2-like receptors were first expressed in whole forebrain sections could be reliably identified but were not significantly different from one another (gestational age 65 for D_1 versus 72 days for D_2); (2) age-related increases in B_{max} for both $[^3H]$-SCH23390 and $[^3H]$-YM09151-2 specific binding could be seen in forebrain; (3) age-related increases in the B_{max} for $[^3H]$- SCH23390, but not for $[^3H]$-YM09151-2, could be demonstrated in cortex; (4) fetal sex was unrelated to the average B_{max} for $[^3H]$-SCH23390 and $[^3H]$-YM09151-2 specific binding in all regions, primarily because of an insufficient male sample size. However, in female forebrain sections, the B_{max} for $[^3H]$-SCH23390 was significantly related to age. Thus, from the middle of the first to the middle of the second trimester, the B_{max} for each ligand increased by an order of magnitude after the onset of the specific binding site's expression in basal forebrain. These data suggest that D_1-like receptor-bearing neurons are more uniformly undergoing age-related increases in expression during gestation regardless of region, whereas D_2-like receptor-bearing neurons fail to show significant age-related increases in cortex. This differential expression of dopamine receptors during development may be a manifestation of different developmental processes (for example, synaptic maturation versus neuronal migration and morphogenesis). These processes may likely have differential developmental outcomes, if disrupted, which may ultimately be relevant to developmental models of early-onset psychopathology.

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A Psychobiological Model of Prenatal Stress: Implications for Fetal Development and Infant Outcome

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A psychobiological model of prenatal stress, fetal development, and birth outcomes is articulated. This model proposes that prenatal stress exerts a significant influence on fetal development and pregnancy outcome, in part, via its influence on the maternal-placental neuroendocrine axis. The model, which is based on an understanding of the ontogeny of fetal development and the endocrinology of human pregnancy, hypothesizes that the effects of prenatal stress are outcome specific and that they are modulated by the nature, timing, and duration of the stressor.

Components of this model were tested in a longitudinal study of more than 375 women with a singleton, intrauterine pregnancy recruited during the second trimester of gestation and followed until 6 weeks postpartum. Structured interviews were conducted to assess prenatal stress and related psychosocial constructs, physical strain, and health-related behaviors. Maternal and cord plasma samples were collected during gestation, delivery, and postpartum for bioassays of stress hormones, including corticotropin-releasing hormone (CRH), adrenocorticotropin hormone (ACTH), β-endorphin (BE), and cortisol. Fetal assessments were conducted at 31–33 weeks gestation, including fetal biometry, biophysical profile, and doppler flow velocimetry of uteroplacental circulation. An experimental paradigm was developed to quantify fetal learning and habituation, assessed by fetal heart rate (FHR) responses to a series of vibroacoustic stimuli.

To date, results indicate that (a) adjusting for parity and antepartum risk, there was a significant prospective association of prenatal stress with infant outcomes including birthweight and gestational age at delivery. (b) Despite the pregnancy-induced activation of the neuroendocrine axis, there were significant cross-sectional associations between maternal psychosocial factors, including prenatal stress and social support, and plasma levels of maternal-placental stress hormones at 28–30 weeks gestation, with psychosocial factors alone accounting for more than 36 and 13 percent of the variance in ACTH and cortisol, respectively. (c) Maternal CRH levels during the early third trimester

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significantly predicted fetal growth (birthweight adjusted for gestational age) as well as the timing of delivery and preterm birth. (d) Preliminary analyses of the fetal heart rate data indicate a significant FHR increase in response to the first few vibroacoustic stimuli with a response decrement to subsequent stimuli, indicating habituation. A two-parameter growth curve (power) model to assess rate of habituation accounted for approximately 70 percent of the variance in FHR response. Small overall differences were detected in habituation rate by fetal sex and by antepartum risk. Analyses are underway to examine individual differences in habituation and their associations with prenatal stress, maternal-placental stress hormones, and outcome. Together, these studies provide important preliminary evidence to support the above psychobiological model. Implications and future directions are discussed.

Early Maternal Separation Alters Neuroendocrine Stress Responsiveness and Central Adrenergic Receptors in Neonatal Rats

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Maternal separation during the early neonatal period (SEP) has important short-term effects on cardiovascular systems, on neuroendocrine regulation, and on behavior. Recently, evidence has been mounting that SEP could also have long-term effects, as it might alter stress responsiveness, cerebral catecholaminergic regulation, and possibly sensitization to substance abuse later during adult life. However, the mechanisms by which SEP could have such long-term consequences are presently unknown. We have demonstrated previously that basal and stress-induced levels of plasma ACTH and corticosterone (B) were increased in 10-day-old (d) pups 24h after SEP, and that this was accompanied by altered adrenal responsiveness to ACTH. Sympathetic activity might mediate at least some of the effects of 24h SEP on adrenocortical activity, since neonatal chemical sympathectomy prevented SEP-induced changes in adrenal sensitivity. We hypothesized that activation of central adrenergic and peripheral sympathetic systems might be partly responsible for some of the long-term consequences of SEP and used 9–10d pups to test whether (1) plasma levels of ACTH, B, and catecholamines were affected at times earlier than 24 h after the beginning of SEP, and (2) 24h SEP affected the abundance of central adrenergic αl and α_2 receptors (AR) by performing autoradiography with either 3H–prazosin or ^{3}H–rauwolscine, respectively. We found that plasma ACTH levels were higher than controls at 3, 6, and 24h after SEP. Plasma B secretion was increased 5-fold over controls 24h after SEP. Plasma norepinephrine and epinephrine concentrations were increased at 3 and 6h, but not at 24h after SEP. However, circulating dopamine was significantly elevated at this latter time point. SEP increased the abundance of α_{2AR} in the dentate gyrus and of α_{1AR} in the hypothalamus of 10-day-old pups, but had no effect of the density of these receptors in either the CA1 region of the hippocampus or the striatum. To verify whether such changes were matched by changes in the abundance of corresponding mRNA transcripts, we performed in situ hybridization for α_{1c} and $\alpha_{1d\,AR}$ mRNAs. Both transcripts were detected in the hypothalami of 10-day-old rats, in contrast to the mRNA for β_{1AR} (detected mostly in the hippocampus at that age).

These results suggest that 24h SEP in 10d pups stimulates both central

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adrenergic and peripheral sympathetic systems, which could maintain tonic activation of the adrenocortical axis. Whether this results in permanent alterations of AR receptor number, turnover, and coupling to second messenger systems is currently under investigation. These studies could provide important insights into the prolonged effects of early neonatal stimulation on stress coping mechanisms and on the development of several mental illnesses, such as depression.

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Development and Plasticity of Visual Memory Circuits

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In adult monkeys, visual recognition memory, as measured by the delayed nonmatching to sample (DNMS) task, requires the interaction between inferior temporal cortical area TE and medial temporal lobe structures (particularly entorhinal and perirhinal cortical areas). Ontogenetically, monkeys do not perform at adult levels of proficiency on the DNMS task until 2 years of age. Several studies have demonstrated that this protracted development of visual recognition memory is due to an immaturity of the association cortex rather than of the medial temporal lobe. For example, lesions of the medial temporal lobe structures in infancy or in adulthood yield profound and permanent visual recognition loss, indicating that the medial temporal lobe structures operate early in life to sustain visual memory. In contrast, early lesions of area TE, unlike late lesions, result in a significant and long-lasting sparing of visual memory ability. Additional evidence for neocortical immaturity was provided by studies of the development of opiatergic and cholinergic receptors, of the maturation of metabolic activity, and of the connectivity between inferior temporal areas TE and TEO and cortical and subcortical structures. Moreover, anatomical studies have shown that early damage to area TE leads to the maintenance of normally transient projections and to the sprouting of additional projections. Together, these results indicate greater compensatory potential after neonatal cortical than after neonatal medial temporal removals. In addition, lesion studies indicate that, during infancy, visual recognition functions are widely distributed throughout many visual association areas but, with maturation, these functions become localized to area TE. Thus, the maintenance of exuberant projections together with reorganization in other cortical areas of the brain could account for the preservation of visual memories in monkeys that have had area TE removed in infancy.

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Innate Recognition of Conspecific Song Is Controlled by Multiple Song Features in Preparation for Song Learning

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The process of song learning is guided by innate predispositions, the elaborate nature of which is only now becoming apparent. It has been shown that young, naive sparrows tutored with tape recordings of both conspecific and heterospecific song preferentially learn conspecific song. We investigated the acoustic and physiological basis for this learning preference using both behavioral and neurophysiological techniques. White-crowned sparrows (wcsp, *Zonotrichia leucophrys nuttalli*), were collected as nestlings and raised by hand in acoustic isolation. In behavioral experiments, we measured the vocal response of fledglings to a variety of song stimuli. These songs included normal wcsp song, "isolate" songs from wcsp raised in acoustic isolation, synthetic songs made by reiterating single wcsp phrases, and songs of sympatric species that wcsp are exposed to in nature. We found that isolate, normal, and synthetically modified wcsp songs were equally potent in eliciting a vocal response in fledglings and were more potent than the songs of other species. Our initial hypothesis was that the birds probably relied on one feature conspicuously present in all natural wcsp songs, namely, the introductory whistle. Instead, we found that they possess an innate ability to make discriminations on the basis not only of the introductory whistle, but also almost all other components of natural song, even when divorced from normal wcsp syntax. To investigate the neurophysiological basis of the innate preference for conspecific song, we recorded single and multi-unit auditory responses of neurons in HVc (the caudal portion of the ventral hyperstriatum or high vocal center) and auditory neostriatum of fledgling wcsp. We found that neurons in these regions responded to distinctive features of wcsp song, some shared with songs of other species, some not. Neurons in these regions did not exhibit a restricted preference for wcsp song, although the basic constituents for such a recognition process appear to be present. We hypothesize that song recognition is accomplished by distributed processing in HVc or in other areas of the brain. Features responded to innately appear to be sufficiently generalized that they permit acquisition of a wide range of local dialects and individually distinctive songs, while restricting the acquisition of heterospecific song.

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Refinement of Intrinsic and Associational Circuitry in Monkey Prefrontal Cortex During Puberty

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Horizontally projecting axon collaterals in the monkey prefrontal cortex arise from and terminate in the superficial cortical laminae in a discrete stripe-like fashion, forming a lattice structure of intrinsic connectivity. Marked decreases in the density of axospinous synapses and dendritic spines during puberty suggest that prefrontal cortical circuitry is substantially refined during this period of development. In this study, we examined whether this refinement process is also reflected in the organization of the lattice structure of intrinsic axon collaterals. Iontophoretic injections of biotinylated dextran amine were made in the superficial layers of areas 9 or 46 in four prepubertal and four young adult macaque monkeys. Tangential reconstructions of anterograde terminal labeling demonstrated that the stripes of the intrinsic lattice were clearly presented in the juvenile animals, but they were 30 percent larger in both length ($p<0.002$) and width ($p<0.01$) than in adults. To quantify the number of axonal boutons and branch points within the intrinsic stripes, all the labeled axons in consistently defined portions of layers I and III were reconstructed. These results revealed a 50-percent reduction in the density of both boutons ($p<0.0001$) and branch points ($p<0.0001$) during puberty in both layers. In order to assess the specificity of these findings, similar analyses of the associational axon collaterals labeled by the same injection sites are being made. Our observations demonstrate that although the intrinsic horizontal circuitry of the prefrontal cortex is established prior to puberty, it undergoes extensive refinement before adulthood. These structural changes may be related to the maturation of cognitive abilities that occur over this period.

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